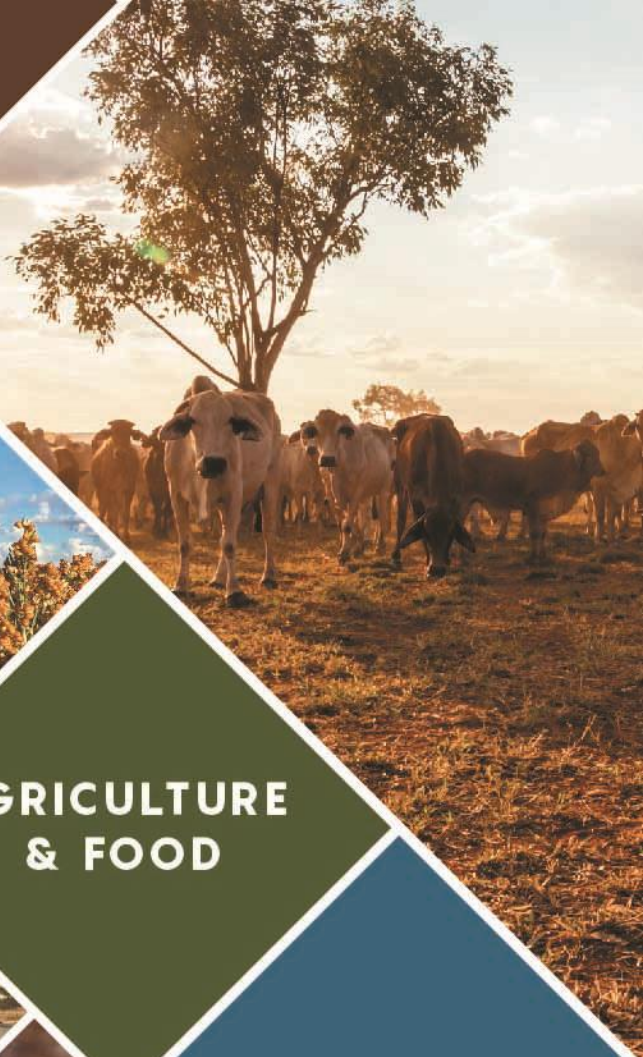
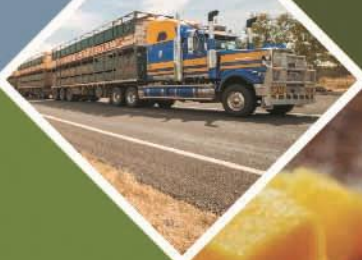
NORTHERN HEALTH SERVICE DELIVERY



TRADITIONAL OWNER-LED DEVELOPMENT

AGRICULTURE & FOOD



Pioneering Tropical Rock Lobster Raft Grow-out for Northern Australia

S. Infante Villamil and J. Blair

Ornatas Research & Development

CRCNA

DEVELOPING NORTHERN AUSTRALIA

Acknowledgements

This research, funded by the CRC for Developing Northern Australia (CRCNA) was supported by the Cooperative Research Centres Program, an Australian Government initiative, and co-funded by the Fisheries Research and Development Corporation (FRDC). The CRCNA also acknowledges the support of its investment partners: the Western Australian, Northern Territory and Queensland Governments.

This report is an output of the CRCNA project "Pioneering Tropical Rock Lobster Raft Grow-out for Northern Australia" a collaboration between Ornatas, Maxima, Honey & Fox, JSJ Seafood, PFG Group, and the University of Tasmania – Institute for Marine and Antarctic Studies. The CRCNA also acknowledges the financial and in-kind support of the project participants.

Disclaimer

Any opinions expressed in this document are those of the authors. They do not purport to reflect the opinions or views of the CRCNA or its partners, agents or employees.

The CRCNA gives no warranty or assurance and makes no representation as to the accuracy or reliability of any information or advice contained in this document, or that it is suitable for any intended use. The CRCNA, its partners, agents and employees, disclaim any and all liability for any errors or omissions or in respect of anything or the consequences of anything done or omitted to be done in reliance upon the whole or any part of this document.

Suggested citation for this report:

Infante Villamil, S. and Blair, J. (2024). Pioneering Tropical Rock Lobster Raft Grow-out for Northern Australia. Final Report CRCNA Project A.3.2021116. Ornatas Research & Development. CRCNA, Townsville. 27 pages.

The CRCNA supports and encourages the dissemination and exchange of its information. The copyright in this publication is licensed under a Creative Commons Attribution 4.0 International (CC BY 4.0) licence.

Under this licence you are free, without having to seek our permission, to use this publication in accordance with the licence terms. You must keep intact the copyright notice and attribute the CRCNA as the source of the publication. For more information on this licence, visit creativecommons.org/licenses/by/4.0.

The information contained herein is subject to change without notice. The CRCNA shall not be liable for technical or other errors or omissions contained herein. The reader/user accepts all risks and responsibility for losses, damages, costs and other consequences resulting directly or indirectly from using this information.

ISBN 978-1-922437-52-5

Australian Government
Department of Industry, Science and Resources

AusIndustry
Cooperative Research Centres Program

Table of Contents

List of Tables

List of Figures

Acronyms

CRCNA	Cooperative Research Centre for Developing Northern Australia
DPIRD	Department of Primary Industries and Regional Development (Western Australian government)
FRDC	Fisheries Research and Development Corporation
HDPE	High Density Polyethylene
IMAS	Institute for Marine and Antarctic Studies (University of Tasmania)
JCU	James Cook University
NT	Northern Territory
ppt	Parts per thousand (commonly used for salinity)
PCR	Polymerase chain reaction
QLD	Queensland
qPCR	Quantitative polymerase chain reaction (where qPCR ct is the cycle threshold value; number of cycles at which the fluorescence signal of the amplified target reaches a detectable level above the background noise, which is a proxy of pathogen quantity in the tested sample)
RA	Risk assessment (refers to Pathogen Risk Assessment)
SCAAH	Sub-committee on Aquatic Animal Health (SCAAH) that provides scientific and technical advice to the Animal Health Committee (AHC). SCAAH comprises representation from the Australian, state and Northern Territory and New Zealand governments, the Commonwealth Scientific and Industrial Research Organisation - Australian Centre for Disease Preparedness (CSIRO ACDP), formerly known as the Australian Animal Health Laboratory, and Australian universities.
SIA	Seafood Industry Australia
TRL	Tropical Rock Lobster (*Panulirus ornatus*)
UTas	University of Tasmania
WA	Western Australia
WP	Work Package
WSSV	White spot syndrome virus

Project Participants

Project Lead Participant

The project received cash and in-kind contributions from – Ornatas

Project Participants

The project was made possible by in-kind contributions from – Maxima; Honey & Fox; JSJ Seafood; PFG Group; University of Tasmania - IMAS

The project was funded by the CRCNA and FRDC

Final Report Authors:

Sandra Infante Villamil and Jennifer Blair – Ornatas Research & Development

Authors of appendices as listed.

Research Contributors:

John Breen, Lachlan Davison, Ben Smith, Harry O'Keane, Caitlin Kelly, Scott Parkinson – Ornatas

Steven Gill, Peter Carrington – Maxima

Jayne Gallagher, Helen Johnston, Joanne Freeman – Honey and Fox

Nathan Maxwell-McGinn – Three Capes Marketing, JSJ Seafood

Peter Warner – PFG Group

Basseer Codabaccus and Greg Smith – University of Tasmania

Leo Nankervis, Nathan Hammel, Kelly Condon – James Cook University – complementary research outside this project

Executive Summary

The Tropical Rock Lobster, *Panulirus ornatus*, demonstrates promise as a valuable addition to the aquaculture sector in Northern Australia, offering both economic and environmental sustainability benefits. Advancements in hatchery and nursery technology have made it feasible to produce juvenile lobsters in land-based commercial scale systems. Hatchery-produced juveniles reduce reliance on wild-caught stocks and alleviate fishing pressure on natural populations. The ability of Ornatas to cultivate Tropical Rock Lobster (TRL) in controlled environments provides the opportunity to optimise growth conditions, leading to higher yields and optimal product quality. Additionally, the establishment of a Tropical Rock Lobster aquaculture industry has the potential to create employment opportunities and stimulate economic growth, particularly in regions where suitable farming conditions exist.

The overall outcomes of this project were to develop and evaluate onshore culture in raft systems and to establish production models for TRL aquaculture in Northern Australia. The research encompassed six work packages. The Farm site environment package investigated the impact of water quality on pilot Tropical Rock Lobster (TRL) production in ponds and pond productivity at the Toomulla site in Queensland. This assessment spanned a full production cycle, from stocking to achieving commercial size and taste testing. Variable environmental conditions were experienced, typical of North Queensland's tropical climate, characterised by seasonal fluctuations between summer and winter. Results indicate that effective water quality management protocols are deemed essential to ensure an optimal growth environment for land-based culture of TRL in North Queensland. The Production system package involved the evaluation and adaptation of an existing offshore Indonesian technology (Aquatech) for land-based TRL culture in Toomulla. Initial testing of an offshore raft system, primarily constructed of High Density Polyethylene (HDPE), was conducted to explore its suitability for potential future TRL grow-out operations in Cone Bay, Western Australia. The third work package focused on biosecurity, translocation, and health, with a primary objective of developing a pathogen risk assessment (RA) framework and protocol for the translocation of hatchery-produced TRL from Queensland to other jurisdictions in Northern Australia. Dr. Ben Diggles led the development of this RA, which serves as the scientific foundation for biosecurity planning, translocation policy, and operational protocols concerning juvenile lobster translocation. Protocols were established for Western Australia and the Northern Territory. The Feeding management package assessed feeding strategies for lobster in raft culture, utilising pelleted feeds formulated by the University of Tasmania (UTas) and hatchery-produced juveniles acclimated to this feed type. The lobster production performance package primarily aimed at developing business models for TRL grow-out operations, supporting opportunities for diversification among existing aquaculture producers, new entrants, and potential investors. Business models tailored for TRL of initial weights 3g and 50g indicated a difference of 3 months to reach commercial harvest size of 1.2kg (14 vs 11 months). Lastly, the market-ready lobster quality package conducted consumer demand research for premium Tropical Rock Lobster to inform ongoing market retention and expansion efforts, encompassing markets beyond China. Additionally, this work package explored technologies related to provenance and branding authenticity.

The project has delivered critical information about production systems, lobster performance, health risks, and demonstrated high quality lobster product from the first production cycle. Based on the results of this research, commercial development and research is continuing in Tropical Rock Lobster aquaculture in land-based raft systems in North Queensland. An industry value of $160 million p.a. and volume of 1,100 tonnes is projected by 2033, employing 120 people in feed manufacture, grow-out, downstream processing/distribution and marketing.

Introduction

The Australian Tropical Rock Lobster (TRL) (*Panulirus ornatus*) is a delicacy in high demand, prized for its excellent texture and flavour, often commanding a premium price (exceeding $80/kg in certain markets). However, the supply of market-size lobsters is currently restricted to wild-caught adults or lobsters grown in Southeast Asia from captured puerulus and juveniles. This places unsustainable pressure on wild TRL stocks, consequently limiting its availability in the medium to long term. The development of novel hatchery technology, regarded as a breakthrough in aquaculture and pioneered in Australia, now enables the production of TRL juveniles. This advancement supports the feasibility of commercialising hatchery-produced TRL and developing a new aquaculture industry.

Ornatas Pty Ltd (hereafter referred to as Ornatas) was founded in 2018 with the explicit goal of pioneering the world's first closed lifecycle Tropical Rock Lobster aquaculture industry for both Australian and international markets. Ornatas holds the Australian license to commercialise the hatchery technology, with the objective of establishing and supporting a grow-out industry in Northern Australia, thereby creating opportunities for diversification among current aquaculture producers. Over the period 2019 - 2029, Ornatas plans to invest more than $65 million in capital to develop this innovative aquaculture sector. Currently, Ornatas operates a commercial scale hatchery, nursery, and a pilot onshore grow-out facility located at Toomulla Beach north of Townsville, Queensland. This comprehensive facility was designed to replicate the oceanic environment necessary for the successful cultivation of spiny lobster larvae and juveniles.

This project, ‘Pioneering Tropical Rock Lobster Raft Grow-out for Northern Australia’, undertook the science required for the grow-out of hatchery-produced juvenile lobsters in raft systems that were trialled onshore at Toomulla. Multiple gaps in knowledge were investigated to support the development and evaluation of this production technology to meet market demands. The project partners brought expertise across the production-to-market pipeline and the research focus was on six areas: environment; raft design; translocation, lobster health and biosecurity; feeding strategies; lobster growth performance; and premium diverse market acceptability. Two business models for an onshore raft system were developed to inform quality investment in a brand-new grow-out aquaculture industry by current and new aquaculture businesses.

All aspects of the research included training of personnel and documentation of procedures that were made available by project participants to potential new lobster grow-out producers in Australia. The project team regularly communicated progress with stakeholders, including government (national, state and territory, local), jurisdiction agencies, community, aquaculture producers, training and research providers. A Field Day event was carried out to share the current status and production models for consideration by existing and potential new businesses. An industry value of $160 million p.a. is projected by 2033, with future potential of over $500 million p.a. that creates 1,000 direct jobs, 900 of those in Northern Australia, for people working in feed manufacture, grow-out, downstream processing/distribution and marketing.

Governance

Across the life of the project, seven steering committee meetings were conducted; two of which took place face to face, one in Broome, Western Australia and one in Townsville, Queensland. The remainder of the meetings were held via Zoom. The final meeting, carried out on May 6th 2024, provided a comprehensive overview of the project, including its accomplishments, encountered challenges, and discussions on future direction.

Steering Committee Members

- Scott Parkinson (Ornatas)
- Jennifer Blair (Ornatas)
- Sandra Infante Villamil (Ornatas)
- Steven Gill (Maxima)
- Jayne Gallagher (Honey & Fox)
- Nathan Maxwell (JSJ Seafood)
- Sarah Docherty (CRCNA)
- Wayne Hutchinson (FRDC)

Invited observers

- John Hutton (Maxima)
- Alison Hutton (Maxima)
- Martin Rees (Ornatas)
- Helen Johnston (Honey & Fox)

In addition to the Steering Committee, Ornatas and Maxima held fortnightly Zoom meetings to share updates and coordinate activities related to the Sea-Raft project. The lead Sea-Raft researchers had a final project update with the CRCNA and FRDC during the end-of-project Field Day, on 13 March 2024 (see Appendix G for a Field Day event Report and Appendix H for the Field Day presentations).

Work Package 1. Farm Site environment

Water Quality Final Research Report (Appendix A) includes a brief review of the environmental conditions in Cone Bay, located within Western Australia's Kimberley region, which was the original designated site for the project. This report also outlines the methodology and water quality findings from the control and grow-out systems at Toomulla throughout the project's duration. The environmental conditions closely reflected those typical of North Queensland's tropical climate, characterised by significant fluctuations between the summer and winter seasons. Summer months, characterised by heavy and frequent rainfall, resulted in decreased salinity levels and high water temperatures. Conversely, the dry winter period led to an increase in salinity levels and cooler temperatures (Table 1). These seasonal variations had a notable impact on overall water quality, often causing deviations from the optimal range for Tropical Rock Lobster (TRL) aquaculture.

Table 1. Total monthly rainfall as per records of the Rollingstone meteorological station (Bureau of Meteorology) and maximum and minimum levels of salinity and temperature from 16 Dec 2022 to 30 April 2024 in the Tropical Rock Lobster grow-out system at Toomulla Beach.

Year	Month	Total monthly rainfall (mm)	Max Salinity (ppt)	Min Salinity (ppt)	Max Temperature (°C)	Min Temperature (°C)
2022	Dec	210.0	34.4	32.4	33.9	28.0
2023	Jan	636.6	32.9	28.8	34.9	29.2
2023	Feb	372.4	28.5	25.9	34.2	28.5
2023	Mar	175.7	30.1	26.1	33.1	27.6
2023	Apr	101.5	33.6	29.7	31.9	25.9
2023	May	8.1	35.4	31.5	28.6	21.7
2023	Jun	2.2	36.0	35.4	26.0	21.7
2023	Jul	78.6	36.1	34.7	25.5	20.8
2023	Aug	3.2	36.1	34.9	25.4	21.9
2023	Sep	18.0	36.8	33.7	27.8	23.6
2023	Oct	15.8	36.6	34.8	28.8	24.1
2023	Nov	40.1	38.1	36.0	30.8	24.1
2023	Dec	112.0	37.2	34.5	31.8	25.6
2024	Jan	416.5	36.1	27.5	32.0	28.3
2024	Feb	547.8	29.6	25.6	31.1	26.4
2024	Mar	323.8	28.1	24.9	31.1	26.4
2024	Apr	45.8	29.9	25.7	29.8	24.4

To maintain suitable conditions for TRL growth, strict daily monitoring and adjustments to water quality were essential (detailed information in Appendix A). During the summer months, the implementation of a cooling tower was necessary to ensure that water temperatures remained below 32°C. Additionally, measures were taken to address the dilution effect caused by heavy rainfall. To counteract this effect, salt or hypersaline water, and sources of calcium and magnesium were introduced into the system. Furthermore, sodium bicarbonate was utilised to raise the pH levels and enhance the buffering capacity of the system. In contrast, during the winter season, the introduction of freshwater was essential to prevent salinity levels from exceeding the tolerance thresholds for TRL. While there was no period of ideal optimal water quality based on available research, throughout the life of this project it was shown that exposure to stressful conditions led to variable levels of TRL survival and growth (detailed in the production performance section; WP5). This study found that a broad range of seasonal temperature and salinity can support TRL survival and growth in North Queensland. Despite the presence of TRL in the system, the low stocking density resulted in minimal nutrient release by the lobsters and gradual decomposition of uneaten feed, indicating a negligible impact of lobster biomass on water quality within the system at the tested stocking densities.

Future developments

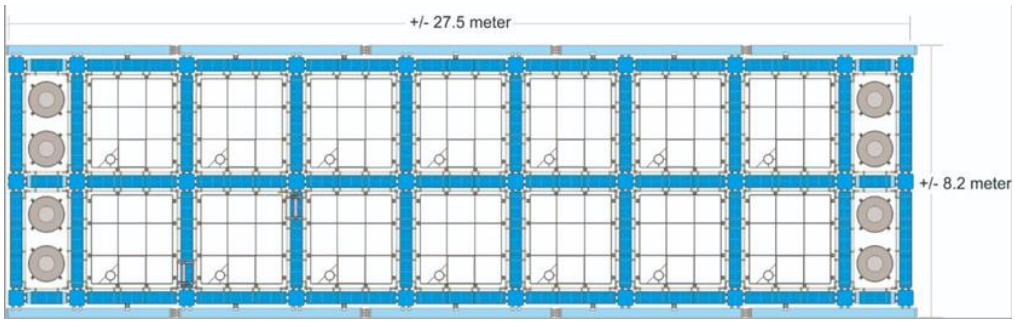
To reach commercial outcomes it is crucial to define levels of key water quality parameters for TRL grow-out to maintain an environment that promotes optimal growth, efficient feed conversion, high survival rates, and overall productivity. Additionally, defining appropriate and tolerance levels allows for the efficient use of resources and ensures the sustainability of operations in the long term.

Work Package 2. Production Systems

The system tested to culture hatchery-produced juvenile TRL in land-based systems at Toomulla Beach consisted of two Aquatec raft prototypes (Figures 1 and 2) with submerged enclosures for communal lobster grow-out. The original Aquatec system consisted of 14 large and 8 small mesh enclosures (Figure 2a). The upper, non-fixed side of each enclosure created an access point for the TRL in the water and this side was used to install the automatic feeder. In the second raft prototype tested the number of enclosures was increased to 16 large and 32 medium enclosures (Figure 2b). Two access points to observe and handle the TRL from the top of the large enclosures were incorporated in the second raft, to facilitate daily activities and feeding. Appendices B and C describe Ornatas' grow-out standard operations and specifications.

Figure 1. Lobster aquaculture raft prototype systems, supplied by Aquatec, Indonesia, for grow-out of juvenile Tropical Rock Lobster in fully submersible mesh enclosures.

a.



b.

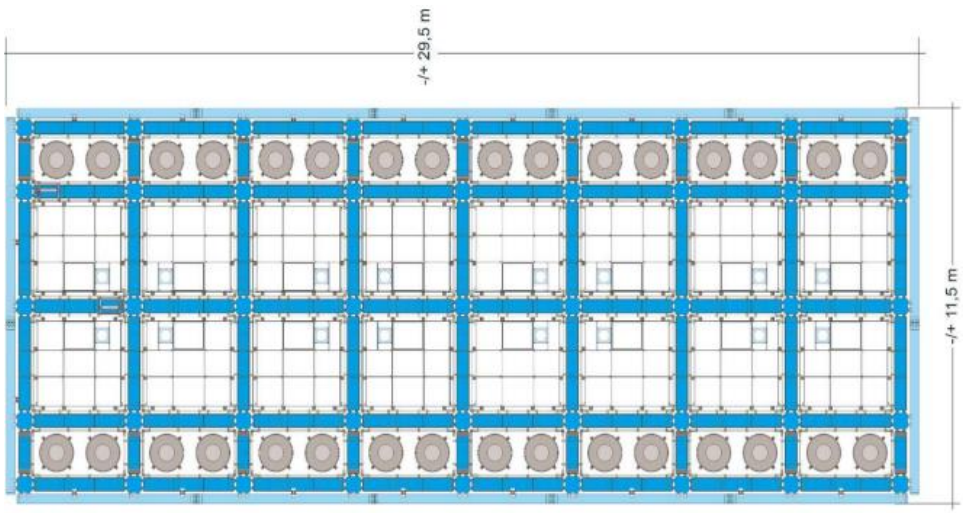


Figure 2. Lobster raft prototype frame system for nursery/grow-out of puerulus and juvenile TRL in submersible structures.

An investigation into the biofouling of equipment in the land-based raft system was conducted, with a particular focus on the mesh of the enclosures. After 22 weeks, heavy biofouling accumulated on the mesh without cleaning (Figure 3). When lobsters were inside the enclosures, internal biofouling was minimal due to the lobsters grazing on fouling organisms. The exterior of the enclosures was brushed clean once every 7 to 14 days to minimize external buildup.

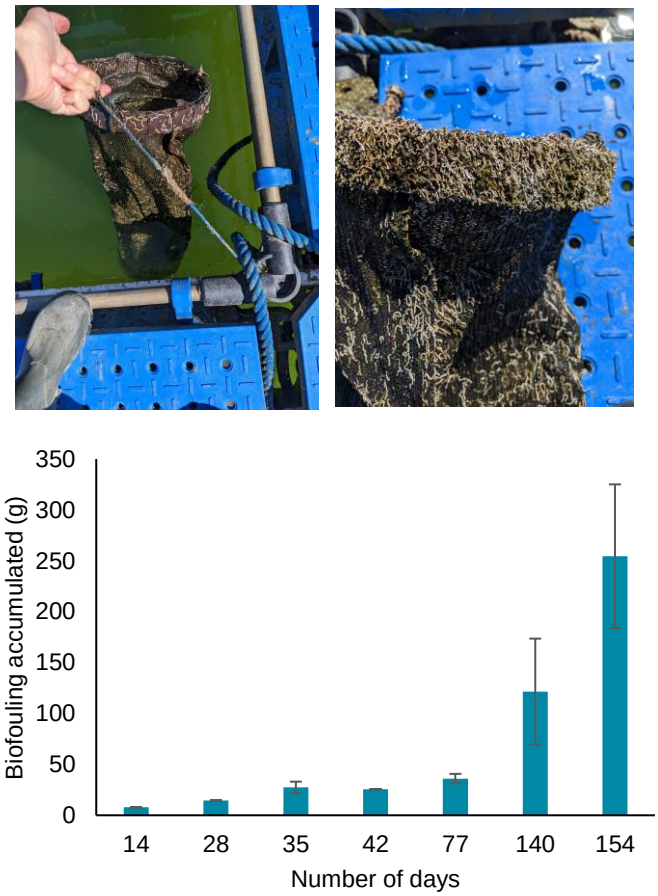


Figure 3. A biofouling trap located in one of the corners of the raft system (top left). A biofouling trap post-deployment showing heavy biofouling accumulated by day 154 (top right). Weight of biofouling accumulation in traps over time (lower graph) without cleaning, average represents average ± SD (n = 2) when available.

In Western Australia, a raft was designed, constructed, and deployed in Cone Bay (Figures 4a–d). However, this raft was not tested with lobster grow-out due to a shift in the project's focus towards research activities in North Queensland.

a.

b.

c.

d.

Figure 4. First sea raft constructed and deployed at Cone Bay.

Future developments

There are several design criteria and operational requirements to consider for improvement of land-based raft operations for TRL production, including: the addition of an automated mechanical track to move around the pen lifting frame and winch; replacement of the small circular pens with rectangular pens for ease of access to lobsters; and, infrastructure to support cooling of pond water during summer (e.g. increased water depth, shading, different lining colour, earthen ponds). Further research is required to investigate scaling with land-based raft production systems, or an alternative technology. Modified systems will be required for nearshore or offshore TRL aquaculture in Northern Australia.

Work Package 3: Biosecurity, translocation and health

The comprehensive risk assessment *Pathogen Risk Analysis for Aquaculture Biosecurity and Translocation of Tropical Rock Lobsters (Panulirus ornatus) in Northern Australia* was developed by Dr Ben Diggles (Diggles, 2021; Appendix D). This risk assessment considered potential risks associated with known exotic and endemic spiny lobster pathogens, originating from the environment and animals housed in land-based systems. Additionally, it assessed the risks posed to existing aquaculture and non-aquaculture species in Northern Australia. The outcome of this risk assessment served as a scientific foundation for developing Ornatas' Biosecurity Management Plan. It also guided translocation policies and operational protocols related to the transportation of juvenile lobsters. The assessment requires regular updates based on scientific literature and experience gained on site and can be utilised for future assessments concerning translocation to Western Australia, the Northern Territory, and/or the Torres Strait, although such endeavours are beyond the current project's scope. A translocation protocol was established through the project for hatchery produced TRL to be sent to Cone Bay, WA, for research purposes (Appendix E). Likewise, a protocol was agreed for translocating juveniles to the Northern Territory into closed culture systems.

As part of this project, a *Health Surveillance and Management Plan* (Appendix F) was developed for Ornatas based on the pathogen risk assessment. This plan is essential to implement surveillance and monitoring initiatives aimed at promptly detecting and effectively responding to disease outbreaks, as well as providing early warnings regarding exotic incursions or emerging diseases.

In collaboration with AquaPath at James Cook University (JCU), and as a component of an Innovation Connections program, an eDNA pilot study was conducted. This pilot project aimed to monitor the bacterium *Aquimarina* sp. within the grow-out system and throughout the site at Toomulla. Figure 5 illustrates the results of bacterial monitoring using eDNA in the grow-out facility during April and May 2023. The findings suggest that *Aquimarina* is a component of the commensal community associated with TRL. While this bacterium has been linked to White Leg Syndrome, and its abundance may indicate bacterial dysbiosis in certain instances (as denoted by a lower qPCR ct value (red line) compared to the 16S value represented by a grey line), no disease was detected in the juvenile TRL held within the grow-out system.

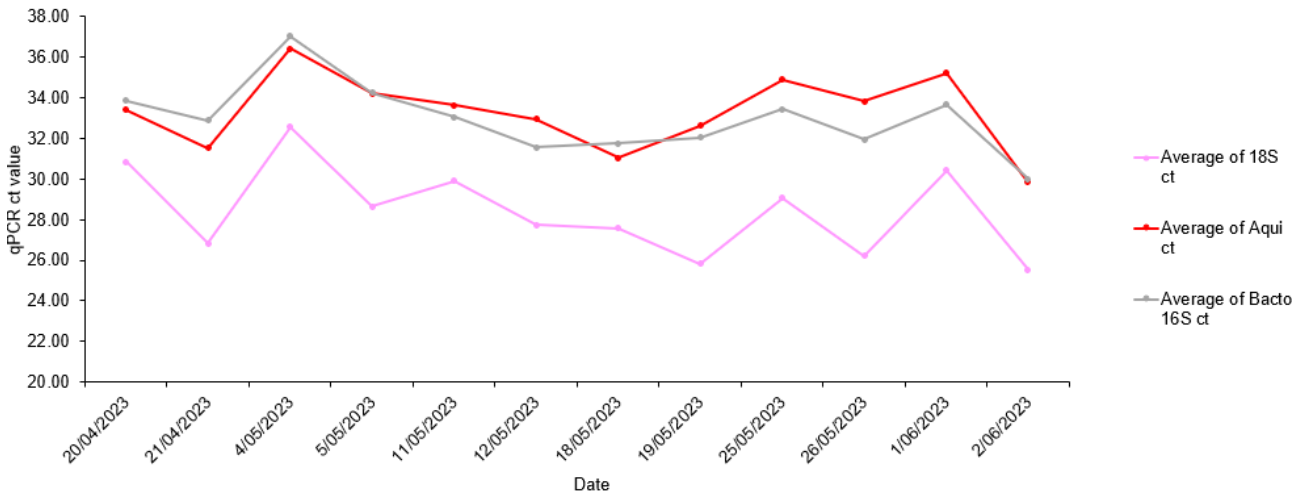


Figure 5. Variation in bacterial load (16S; grey line), *Aquimarina* load (red line) and *P. ornatus* load (pink line) based on qPCR results.

We acknowledge the contribution of Kelly Condon and Maria Andrade-Martinez towards this work package through eDNA research conducted. Suggested citation for this section:

Infante Villamil, S., Condon, K., Andrade-Martinez, M. and Blair, J. (2024). Work Package 3. Biosecurity, translocation and health. In Infante Villamil, S. and Blair, J. (2024). *Pioneering Tropical Rock Lobster Raft Grow-out for Northern Australia. Final Report CRCNA Project A.3.2021116*. Ornatas Research & Development. CRCNA, Townsville. 27 pages.

Future developments

Establishing a new tropical rock lobster aquaculture industry in Northern Australia requires continuous progress in the areas of biosecurity, translocation, and health management. Biosecurity protocols must undergo constant review and adaptation to address new and emerging disease threats and changes in farm conditions, including the scaling up of operations. Assessing the health of lobsters and evaluating methods to prevent and manage potential pathogens in TRL juveniles is paramount for ensuring the success and sustainability of the industry. This involves ongoing passive surveillance and prompt response to any moribund or unusual signs and symptoms observed among the juvenile lobsters, including changes in behaviour, lesions, or unusual morphology. Additionally, active surveillance and sample testing should be conducted for all incoming new broodstock, with a particular focus on PCR testing for white spot syndrome virus (WSSV). Conventional methods, such as routine health examinations and quarantine procedures, can be enhanced by innovative strategies like microbial monitoring and management, including the utilisation or development of probiotics. Probiotics have the potential to provide health benefits to the host, such as reducing the risk of opportunistic bacterial proliferation.

Work Package 4. Feeding management

Feeds developed by the University of Tasmania (UTas) through the ARC Research Hub for Sustainable Onshore Lobster Aquaculture underwent evaluation in the sea rafts. It is important to note that these feeds are protected under the intellectual property rights (IP) of both UTas and Ornatas, as part of their research collaboration background.

Feeding management is described in Appendix B (Best Practice grow-out operation). Briefly, automatic 12-h belt feeders (Figure 6) were used to dispense feed three times per day in the individual enclosures. The feed ration was calculated based on 2% of the initial lobster biomass per enclosure followed by adjustments based on observations of feed not consumed and weekly counts of lobsters in each enclosure. These observations in individual pens relied on optimal water quality to avoid undue stress to TRL and were dependent on water clarity to allow counting. Underwater video surveillance was carried out to observe feed attraction and consumption but was limited to periods of adequate visibility.

Figure 6. Belt feeders installed to medium size enclosures in the grow-out system.

As part of an Innovation Connections program with JCU, research in hydroacoustics was conducted to address visibility limitations and optimise feeding efficiency in the grow-out systems. This research is ongoing and can improve feeding by providing real-time monitoring and assessment of feeding behaviour. The main goal is to adjust feeding protocols to ensure the right amount of feed is delivered at the right time. This optimisation can lead to improved growth rates and minimise the risk of overfeeding, which can lead to water quality degradation. In the long term hydroacoustic monitoring can help identify abnormal feeding behaviour or changes in feeding patterns, which may indicate health issues or stress in TRL.

Hydroacoustic research started in Nov 2023 using an automated belt feeder, a hydrophone and an underwater video camera to precisely record and identify TRL feeding activity. Accuracy in feeding time was limited due to the design of the belt feeder. However, three different sounds produced by TRL were recognised: popping, rasping and slow rattle sounds. Throughout a 3-hour period around delivery of feed in a raft enclosure, the popping sound was the most common of the three sounds. This sound is generated through the motion of a lobster's appendages and the cavitation bubble mechanism during feeding. The "popping" sound can occur either as a solitary pulse (see Figure 7) or in clusters of up to three pulses (Figure 8).

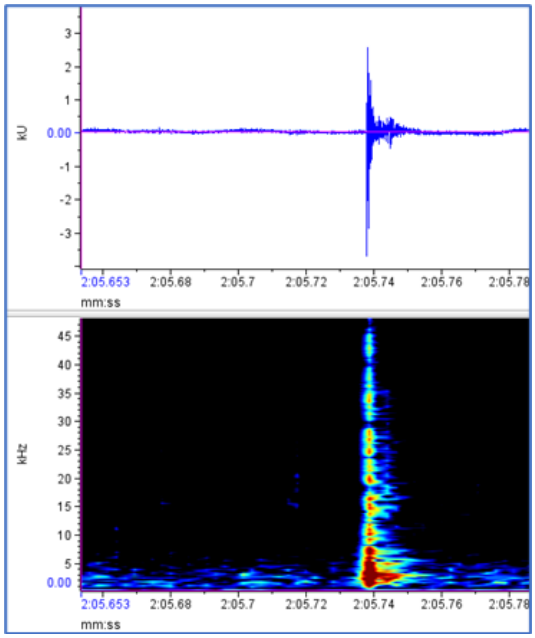


Figure 7. Popping sound of a tropical rock lobster as a singular pulse. The top quadrant shows the waveform view and the bottom quadrant shows the spectrogram view of the hydrophone recording.

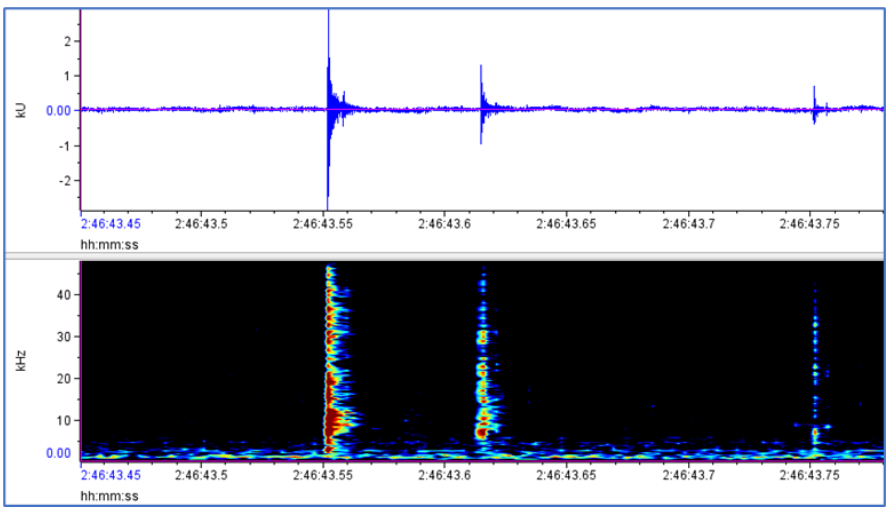


Figure 8. Popping sound of a tropical rock lobster as a group of pulses. The top quadrant shows the waveform view and the bottom quadrant shows the spectrogram view of the hydrophone recording.

During the 3-hour recording, 399 popping sounds of broad levels of amplitude and frequency were recorded. These broad levels could be associated with feeding background noise from TRL located in other enclosures within the raft system and TRL of different sizes. Figure 9 shows the number of popping sounds recorded by the hydrophone in 10-minute intervals. The highest number of “popping” sounds (n=40) was recorded during the 80 to 90 min interval after the first feeding. The second highest number (n=35) was recorded during the first 10 min after the first feed (Figure 9). If all the popping sounds recorded were from TRL in the same enclosure, results suggest that the animals fed at different times throughout the 3-hour tested (~15:00 – 18:00).

The rasping sound was less common than the popping sound and it had a longer duration and lower amplitude and frequency. This sound was detected 75 times in the 3-hour recorded. The highest number of rasping sounds was recorded in the 60 – 70 min interval after the first feed (Figure 10). It has been suggested that the emission of the rasping sound in lobsters occurs in response to perceived threats or

disturbances in their environment. Hartoyo et al., (2022) proposed that this sound escalates in frequency during nighttime hours due to heightened sensitivity to environmental movement. Additionally, the rasping noise has been identified as a defensive mechanism against potential predators (Buscino et al. 2011). Furthermore, research conducted by Staaterman et al. (2010) indicates that simulation of contact with a predator can readily provoke this sound in lobsters.

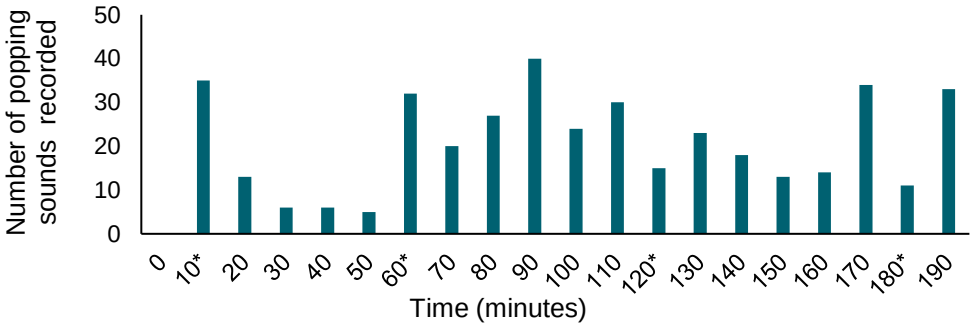


Figure 9. Number of popping sounds produced by tropical rock lobsters recorded by the hydrophone grouped by 10-minute intervals. "*" beside x-axis labels indicates feed input times.

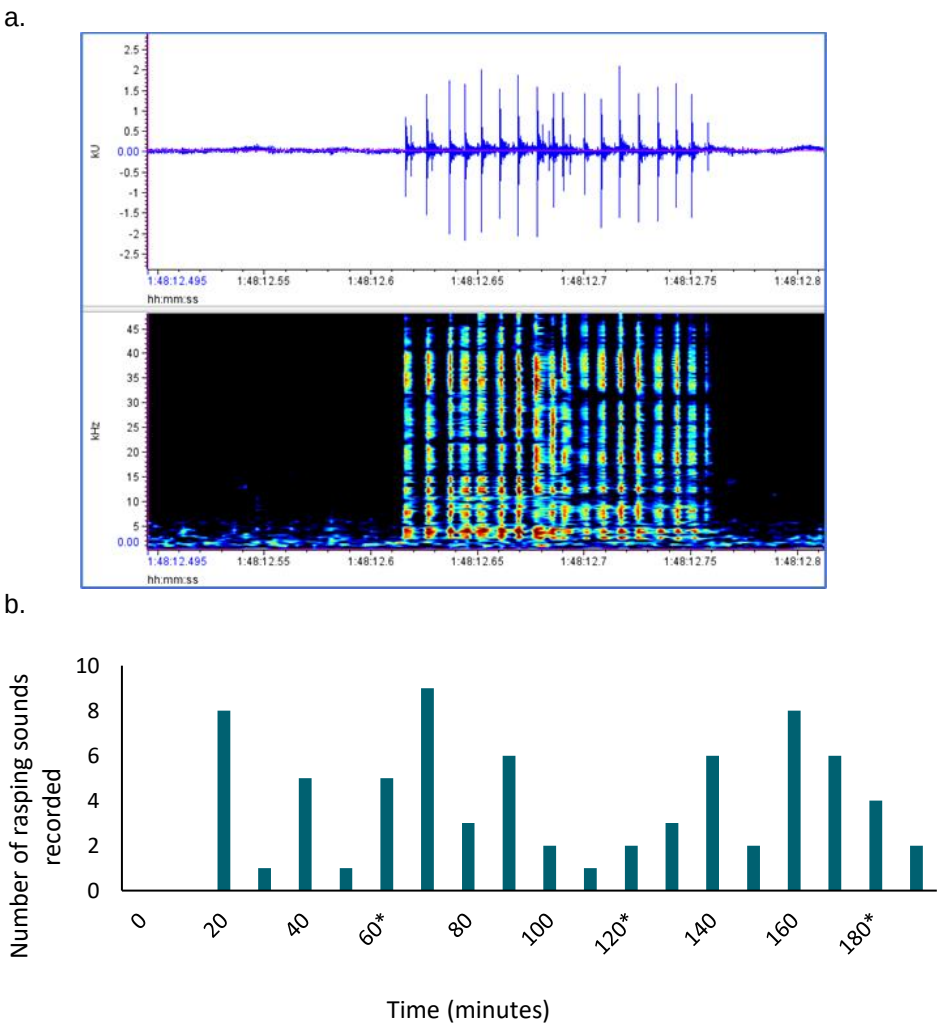
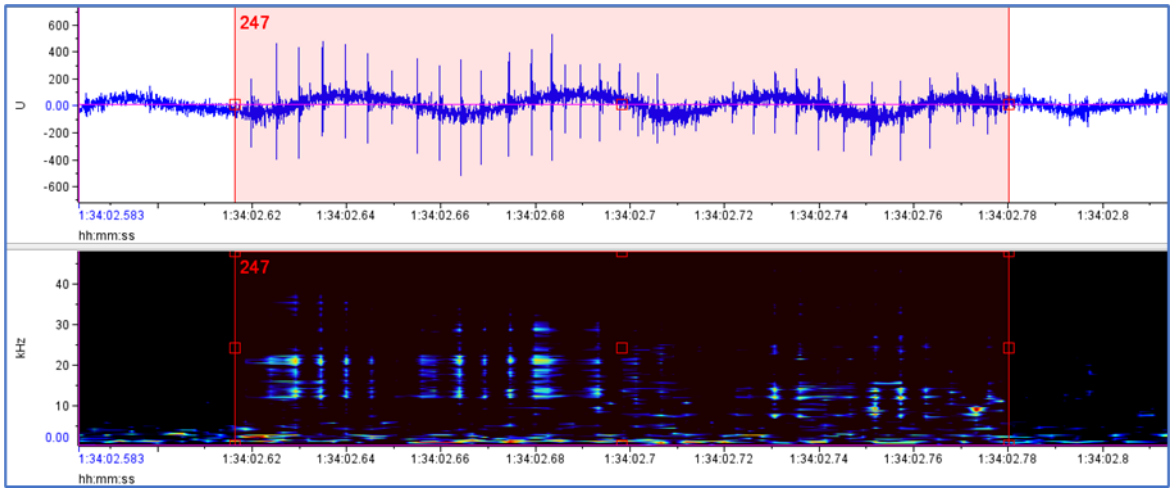


Figure 10. Waveform view (top quadrant a) and spectrogram view (bottom quadrant a) of the rasping sound produced by tropical rock lobsters. (b.) Number of rasping sounds recorded by the hydrophone grouped by 10-minute intervals after feeding. "*" beside x-axis labels (b) indicates feed input times.

The slow rattle sound is the least understood and it is believed to be produced in the same way as the rasping sound (Hartoyo et al., 2022). It was detected 25 times during the 3 hours of monitoring (Figure 11).

a.



b.

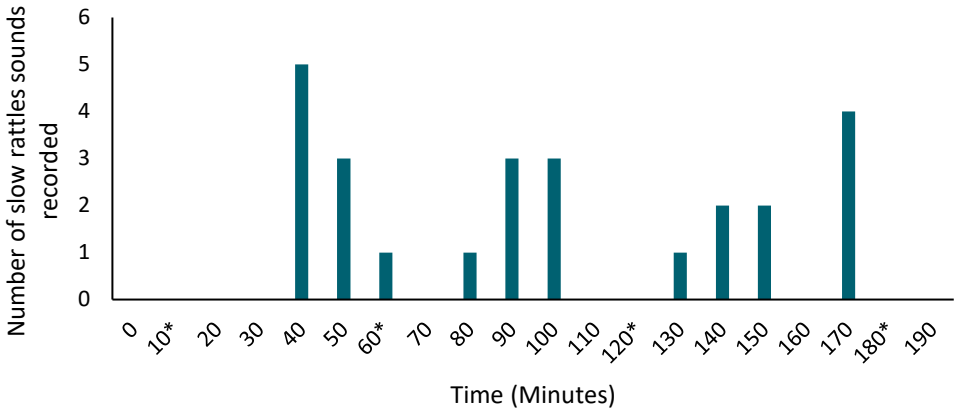


Figure 11. Waveform view (top quadrant a) and spectrogram view (bottom quadrant a) of the slow rattle sounds recorded that were produced by tropical rock lobsters. Number of slow rattle sounds recorded by the hydrophone grouped by 10-minute intervals after feeding. "*" beside x-axis labels (b) indicates feed input times.

A subsequent experiment was carried out to overcome the constraints observed in the previous trial. Firstly, an Arvo-Tec automatic feeder (Figure 12) was tested due to its capability to deliver precise feeding times and quantities. Secondly, the experiment was conducted in the second grow-out system, where lobsters were segregated within a single enclosure positioned at the far end of the system, away from the paddlewheel. The outcomes of this trial are pending analysis and will inform future research direction.

Figure 12. Arvo-tec feeding system installed to a large enclosure in the second grow-out system to increase accuracy in feeding time and volume.

We acknowledge the contribution of Nathan Hammel and Leo Nankervis towards this work package through hydroacoustic research conducted. Suggested citation for this section:

Infante Villamil, S., Hammel, N., Nankervis, L. and Blair, J. (2024). Work Package 4. Feeding Management In Infante Villamil, S. and Blair, J. (2024). *Pioneering Tropical Rock Lobster Raft Grow-out for Northern Australia. Final Report CRCNA Project A.3.2021116*. Ornatas Research & Development. CRCNA, Townsville. 27 pages.

Future developments

Feeding practices need to be continuously optimised to ensure efficient TRL growth and sustainability of production by avoiding feed waste. One key priority is the continued development and implementation of hydroacoustic technology along with automated feed delivery, to better align feeding regime with lobster feeding response and to minimise waste and potential water quality deterioration (potential environmental impact). Additionally, the development of a commercial feed through the exploration of alternative feed ingredients and formulations is ongoing, to improve feed efficiency and reduce production costs.

Work Package 5. Lobster production performance

The processes and parameters used to evaluate TRL performance in the grow-out system are described in Appendix B. Weight and survival based on weekly counts were the key parameters used to determine TRL performance by season and to develop the business model. A total of 886 hatchery-produced juvenile lobsters were stocked in the grow-out facilities between December 2022 (Group A only in Table 2) and February 2024, and reared until April 2024. Table 2 provides a summary of the fluctuations in weight and survival rates observed during the stocking events of hatchery-produced TRL across different groups throughout the duration of the project. The lowest survival rate (22.7%) was observed in Group A1, corresponding to the initial stocking event of the project. This poor survival rate is likely attributed to environmental parameters fluctuating beyond the tolerance level for Tropical Rock Lobsters (TRL). Specifically, high temperatures ranging from 33.1 to 34.9 °C, alkalinity below 2.5 Meq/L, pH levels below 8.1 with significant diurnal fluctuations due to low buffering capacity, magnesium levels below 1320 ppm and calcium levels below 380 ppm (refer to Table 1 and Appendix A). Survival rates equal to or higher than 40% were observed in the remaining groups (A2 – A8 and B1 – B2), with over 90% survival in the batches stocked in 2024.

Table 2. Summary of TRL performance in grow-out rafts at Toomulla Beach throughout the project.

Group	Date 1	Date 2	Initial wt (g)	SD	Final wt (g)	SD	Survival
A1	4/01/2023	27/03/2023	64.26	23.4	85.3	15.5	22.7
A1	30/11/2023	26/02/2024	530.7	74.0	663.5	177.5	**84.6**
A2	30/03/2023	12/05/2023	40.2	5.0	76.35	9.1	**61.3**
A2	12/05/2023	6/09/2023	76.35	9.1	228.0	47.9	**71.4**
A3	12/04/2023	15/06/2023	6.0	1.8	25.9	9.9	50.0
A3	12/04/2023	15/06/2023	7.2	1.4	23.7	10.8	46.7
A3	12/04/2023	15/06/2023	6.5	2.1	32.7	8.8	40.0
A3	15/06/2023	14/09/2023	27.1	14.2	99.0	50.0	**58.5**
A4	12/04/2023	15/06/2023	33.4	5.6	82.6	13.7	43.3
A4	12/04/2023	15/06/2023	32.3	3.4	77.6	16.8	50.0
A4	12/04/2023	15/06/2023	31.5	5.3	70.2	14.8	**60.0**
A4	15/06/2023	14/09/2023	76.1	15.7	178.3	42.1	**73.9**
A5	20/04/2023	16/05/2023	20.5	2.6	34.03	16.01	**76.8**
A5	16/05/2023	6/09/2023	34.03	16.01	125.8	41.6	**68.3**
A5	20/04/2023	16/05/2023	46.4	19.9	104.98	23.06	**92.0**
A5	16/05/2023	6/09/2023	104.98	23.06	265.6	75.8	**60.9**
A6	7/06/2023	22/08/2023	2.6	0.8			95.0
A6	7/06/2023	22/08/2023	2.6	0.8	23.1	9	47.4
A6	7/06/2023	22/08/2023	2.9	1.2			100.0
A6	7/06/2023	22/08/2023	2.9	1.2	19.7	9.0	45.0
A6	7/06/2023	22/08/2023	5.7	1.6			90.0
A6	7/06/2023	22/08/2023	5.7	1.6	32.3	12.6	**55.6**
A6	7/06/2023	22/08/2023	5.4	2.1			95.0
A6	7/06/2023	22/08/2023	5.4	2.1	32.3	18.7	47.4
A6	7/06/2023	22/08/2023	3.5	0.8			85.0
A6	7/06/2023	22/08/2023	3.5	0.8	23.4	5.5	**64.7**
A6	22/08/2023	30/11/2023	26.2	12.3	78.9	35.7	**89.6**
A6	30/11/2023	25/04/2024	78.9	35.7	317.4	94.2	**69.8**
A7	15/11/2023	24/4/2024	9.39	_	122.1	19.46	**86.0**
A7	15/11/2023	24/4/2025	6.83	_	104.68	22.11	**80.5**
A7	16/11/2023	24/04/2024	8.94	2.88	135.5	28.2	**96.0**
A7	16/11/2023	23/04/2024	8.07	2.23	121.06	21.46	**88.0**
A8	21/12/2023	25/04/2024	61.74	18.4	305.53	103.2	**78.8**
B1	8/02/2024	25/04/2024	45.15	14.42	105.6	24.11	**96.2**
B2	8/02/2024	25/04/2024	42.16	7.29	103.04	26.59	**96.3**
B2	8/02/2024	25/04/2024	38.08	10.05	97.33	17.64	**92.3**

A replicated size-at-stocking experiment involving TRL subjected to winter conditions (Groups A3 and A4), revealed that individuals of small (~6.6 g) and medium (~32.4 g) sizes stocked in medium enclosures (0.61 m^2) demonstrated resilience and growth even at salinity levels exceeding 35 ppt (with a maximum of 36.0 ppt observed in June) and temperatures as low as 21.7 °C recorded in May and June (refer to Figure 13, Table 2, and Appendix A). By the conclusion of the 2-month experiment in mid-June 2023, survival rates for these groups approached 50%. Notably, following the consolidation of individuals from each size group from the

medium enclosures into larger ones (6.75 m² each) in June, improved survival rates were observed in the larger size group (73.9%) compared to the smaller size group (58.5%) by mid-September. Throughout this period, the TRL experienced winter conditions, enduring temperatures as low as 20.8 °C in July and salinity levels as high as 36.8 ppt in September (see Table 2). It is plausible that smaller animals may exhibit increased cannibalistic behaviour or be less tolerant to such challenging environmental conditions, resulting in lower survival.

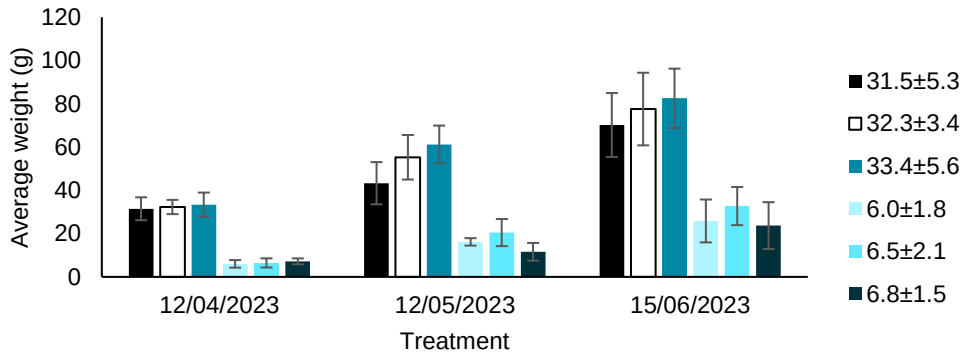


Figure 13. Variation in weight of TRL exposed to winter conditions in the grow-out system (size at stocking experiment; Groups A3 and A4). Legend indicates initial average size of lobsters in grams in each enclosure, small lobsters (6.0 to 6.8 g) and medium lobsters (31.5 to 33.4 g).

In a second replicated trial involving 100 animals, distributed across five medium enclosures (Group A6), approximately 50% survival was confirmed for small-sized animals during winter conditions (June 7th to August 22nd). The TRL weighed on average between 2.6 and 5.7 g, resulting in a survival rate of 52.0 ± 8.2%. Additionally, this trial revealed that 7% of mortality could be attributed to the stress associated with stocking, which involves the processes linked to the movement of animals from the nursery into the grow-out system. These two winter experiments highlight a limitation in stocking small-sized animals (approximately 32.0 g or smaller) during winter conditions in the tested land-based system, with only an estimated 50% survival rate among the stock. Upon transferring the surviving TRL into a large enclosure on August 22nd, with an average weight of 26.2 ± 12.3 grams, survival reached 89.6%, despite the group being exposed to the highest salinities of the year in November (reaching 38 ppt by the end of November). In addition, temperatures were warmer, ranging from 23.0°C in late August to 30.8°C in late November.

The highest survival rate achieved during the project (averaging 88%) occurred during the second summer season in 2024, as evidenced by a trial involving TRL smaller than 10 grams (Group A7; Table 2). Despite being exposed to high salinities in November (maximum 38.1 ppt), the addition of freshwater and the onset of rainfall events led to a decrease in salinity, with a maximum of 29.9 ppt recorded in April and a minimum of 24.9 ppt in March. Several factors may have contributed to this improvement, including a reduction in stocking density (55 or 75 TRL in large enclosures), warmer but not extreme temperatures ranging from 25.3°C in November to a maximum of 32.0°C in January (attributable to the effect of the cooling tower), and alkalinity and pH levels fluctuating within tolerance levels (refer to Appendix A). Conducting a summer stocking experiment with higher stocking density and using managed water quality conditions (implemented as standard procedure from March 2023) would serve to validate these promising results.

A business model based on the assumptions of no impact of the season on growth, and a worst-case scenario of 50% cannibalism from stocking, indicates that 14 months are required for TRL of initial weight 3 g to reach the commercial harvest weight of 1.2 kg. The model predicts 3 months less (11 months) for animals of initial weight 50 g to reach harvest size. Figure 14 indicates the change in biomass over time with these assumptions and starting from either 50 g or 3 g juvenile TRL. Research is ongoing to determine methods to mitigate cannibalism in grow-out.

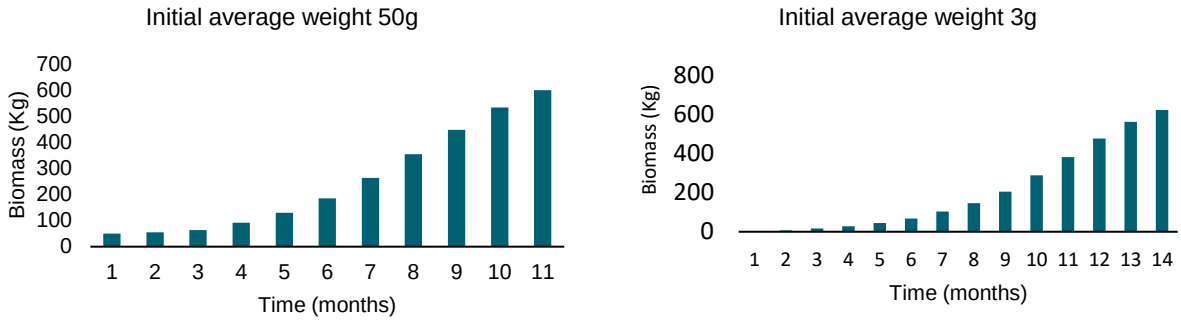


Figure 14. Business models developed for TRL of two initial sizes of juvenile Tropical Rock Lobsters based on growth and survival data gathered in the grow-out system.

Field Day

A Field Day was held on the 13th of March 2024. Participants from CRCNA including board members and Sarah Docherty (SC), FRDC, AusIndustry, JCU, IMAS, Office of Northern Australia, Steven Gill (SC Maxima), Ornatas SC (Jennifer Blair and Sandra Infante Villamil), Ornatas' General Manager and Nursery and Grow-out Manager. The main goal of the Field Day was to disseminate knowledge gained by Ornatas throughout the life of the Project on the development, current status and production models of the TRL aquaculture industry. The outcomes of the project were evaluated and the research needs and opportunities to develop the industry in Northern Australia were discussed. Refer to Appendix G for details of the Field Day and to Appendix H for the information shared with all participants.

Future developments

Continued monitoring of growth throughout a complete production cycle with adjusted water quality is required. Although the current water quality may not be optimal for Tropical Rock Lobster (TRL) growth, the collected data will demonstrate the effects of cost-effective adjustments and water quality (WQ) management on productivity.

Continued research and development efforts aimed at optimising stocking density and refining feeding strategies to enhance productivity are warranted.

There is the prospect of assessing the feasibility of TRL grow-out in nearshore ocean environments that provide different water quality conditions to the land-based site tested in this project. Opportunities in WA, where baseline water quality data is available, and the NT or Torres Straits could be explored to determine the suitability and potential for TRL aquaculture initiatives.

Work Package 6. Market-ready lobster quality

This work package comprised several approaches. It monitored the market and evaluated market demands by establishing a market monitoring system and conducting consumer demand research for premium Tropical Rock Lobster, which informs market retention and expansion efforts. Conducting demand research was crucial for informing the market acceptability of lobsters produced in sea rafts. This is particularly important as there was no prior information about the product appearance (e.g. potential for external biofouling), or flesh quality in hatchery-produced lobsters grown on a formulated feed.

Appendix I provides a summary of WP6 outcomes. Briefly, six markets were identified as suitable candidates for Ornatas to implement a diversified market development strategy, particularly as production scales up. These candidates are China, Hong Kong, the USA, Singapore, Taiwan and Korea. State-of-the-Market Reports are commercial in confidence. Market demand research included Chinese importers and Consumers. For Chinese importers, robustness (survival in tanks upon arrival) is the most important quality of live lobsters. Findings on modern Chinese consumers are detailed in Appendix J. This appendix includes people's perception of lobsters in general, a description of the most popular retailers, typical consumer profiles and purchase channels. Research conducted by Daxue, a specialist Asian market research agency, highlights the difference between Chinese, Korean and Singaporean lobster consumers (Appendix K).

Research on technologies related to provenance and branding authenticity is ongoing, beyond this project, and current options were captured on the *Provenance Technologies Report* (Appendix L).

This work package also evaluated onshore-grown lobster quality and market requirements by conducting an internal taste testing and a farm tour and taste testing with Australian chefs, wholesalers and retailers (Appendix M). Both experiences highlighted the high quality of the hatchery-produced product. Externally, there was no evidence of biofouling on the lobster carapace, which enhanced the appearance of the animals and the whole tasting experience. The experts characterised the hatchery-produced lobsters as robust, full of liveliness and with a clean taste. Different dishes were prepared with the no-waste goal; the tested product performed well in all circumstances.

Future developments

In relation to TRL quality and market demand, there are several areas for further research and development:

Emphasising the importance of animal survivability and robustness upon arrival to meet buyer expectations, it is important to develop harvesting, packing, and transportation capabilities for both live and dead products. Collaborating with businesses experienced in lobster export is key to leveraging their expertise and facilities.

Collaborate with chefs who participated in the taste testing to investigate various product formats and create a best practice guide to handling and cooking TRL using the 100% product utilisation approach. Carry out a second testing trial that includes the response of the chefs, the buyers and the consumers.

Maintain market monitoring and begin with market education to position the hatchery-produced TRL as a “world first”. An engaging product provenance story can be created emphasising sustainability, premium quality, Australian origin, and delicious taste. Market education efforts should be evaluated and provenance technologies such as QR codes and track and trace, should be tested.

Develop a market entry strategy in the local region while the volume of production increases and supply is continuous. Price premiums are likely to be achieved with small volumes of product if correct handling is coupled with premium branding and market positioning. A transition into a phased expansion can start with premium markets in Brisbane, Sydney, and Melbourne. This market strategy allows Ornatas to grow, establish processes, and train team members, supply chain and market partners. Once export to China is open for TRL, product testing in different Chinese markets can be explored.

Strategic recommendations

Key priority actions for sector development	Action owner and key partners	Pathways to implementation and timeline	Intended industry impacts
Additional research is required to support Tropical Rock Lobster grow-out aquaculture in Northern Australia. Priorities are: 1. establish an economical, commercially manufactured formulated feed, 2. investigate scaling land-based raft production systems, or alternative technology, 3. assess the health and management of potential pathogens of lobster juveniles, 4. define appropriate water quality for grow-out, and 5. test grow-out in nearshore ocean conditions.	**Ornatas**	Ornatas plans to continue to invest in collaborative research in TRL aquaculture, and has commenced discussions with potential funding agencies, industry stakeholders, and research providers.	Ornatas is scaling production with a goal of 1,100 tonnes p.a. of premium lobster product by 2033. This scale represents $160 million p.a. GDP, 120 jobs, and regional employment in Northern Australia, which includes opportunities for Indigenous-led businesses.
Streamlining translocation requirements for aquaculture species, including hatchery-produced and wild caught Tropical Rock Lobsters, within and between state jurisdictions - specifically harmonising cross-border biosecurity planning.	**Sub-committee on Aquatic Animal Health (SCAAH)** FRDC SIA	A workshop was hosted by SIA on 5 July 2023 with input from government and industry, including a presentation on TRL. A project identifying improvements to the translocation process for abalone, oysters and prawns is underway (supported by FRDC and SCAAH). Activities in other sectors to be progressed.	To manage the risks of pathogen spread and potential for disease outbreak and make the translocation process more efficient for industry and government agencies, with an agreed approach for each species/sector that is consistent across jurisdictions.

References

Buscino, G., Filiciotto, F., Gristina, M., Bellante, A., Buffa, G., Di Stefano, V., Maccarrone, V., Tranchida, G., Buscaino, C., and Mazzola, S. (2011). Acoustic behaviour of the European spiny lobster *Palinurus elephas*. *Marine Ecology Progress Series*, 441: 177-184.

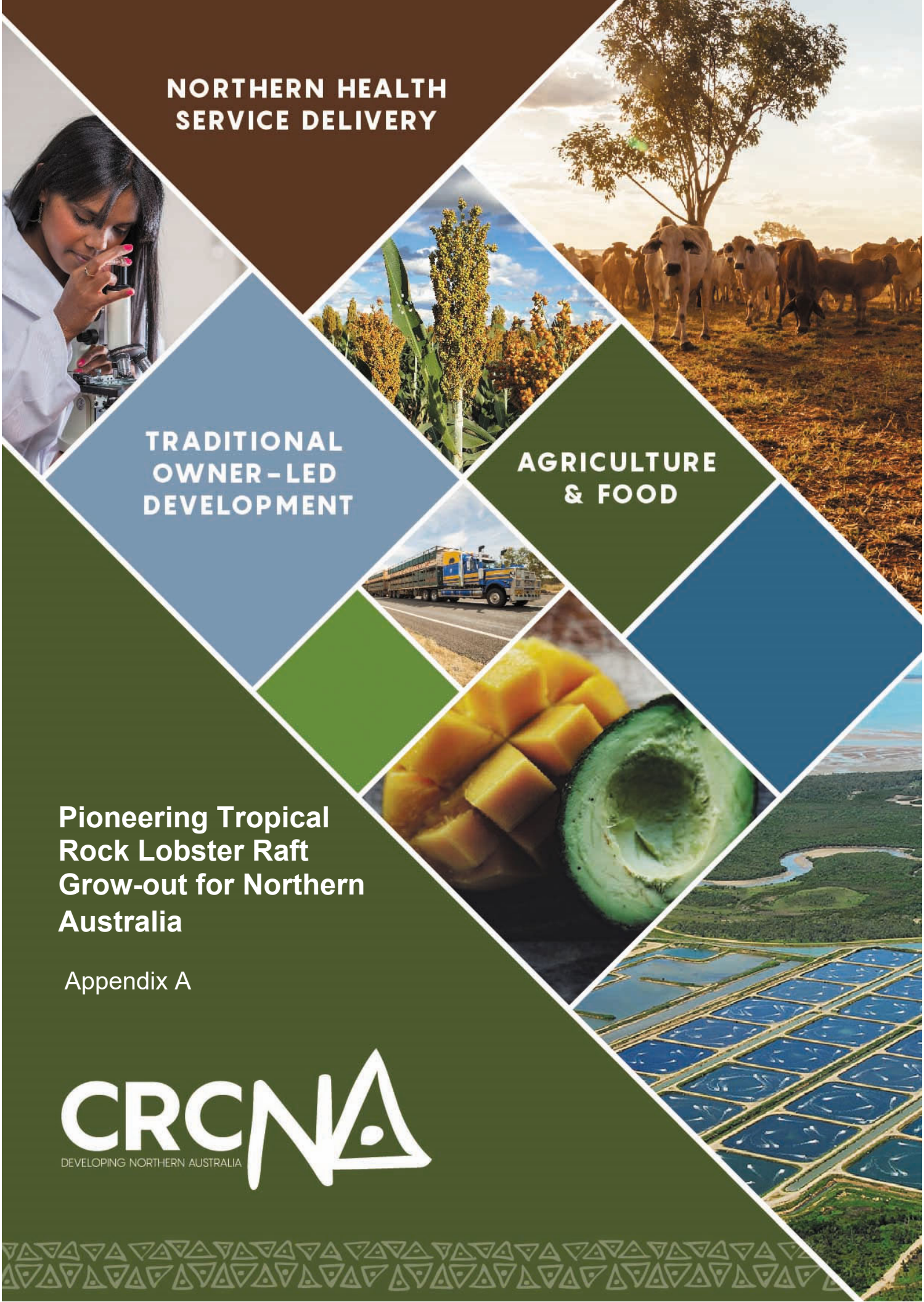
Diggles, B.K. (2021). Pathogen risk analysis for aquaculture biosecurity and translocation of tropical rock lobsters (*Panulirus ornatus*) in Northern Australia. *DigsFish Services Report DF21-03b*, 29 November 2021, 120 pgs.

Hartoyo, H., Amron, A., Fitri, A.D.P., and Darmanto, Y.S. (2022). Sound productivity of spiny lobster *Panulirus homarus* (Linnaeus, 1758) due to crude oil contamination: the basic of bio-acoustic identification for water quality purposes. *Omni-Akuatika*, 18: 72-78.

Staaterman, E.R., Claverie, T., and Patek, S.N. (2010). Disentangling defense: The function of spiny lobster sounds. *Behaviour*, 147: 235-258.

Appendices

Appendix A	Water Quality Report 5 – Water quality variability Dec 2022 to April 2024 in TRL grow-out
Appendix B	Ornatas Best Practice grow-out operation
Appendix C	Ornatas Grow-out Standard Operating Procedure
Appendix D	Pathogen Risk Analysis for Aquaculture Biosecurity and Translocation of Tropical Rock Lobsters (*Panulirus ornatus*) in Northern Australia
Appendix E	Health Protocol for the Import of TRL Juveniles into WA (DPIRD)
Appendix F	Ornatas Health Surveillance & Management Plan
Appendix G	Field Day Event Report – 13 March 2024
Appendix H	Field Day Presentations
Appendix I	WP6 Market Ready Lobster Quality
Appendix J	Modern Chinese Consumer Research Report
Appendix K	Preference survey for lobster consumers in China, Singapore and Korea
Appendix L	Provenance Technologies Report
Appendix M	Farm Tour Taste testing



Pioneering Tropical Rock Lobster Raft Grow-out for Northern Australia

Appendix A

CRCNA
DEVELOPING NORTHERN AUSTRALIA

WP1 Water quality report No. 5

CRCNA: Pioneering Tropical Rock Lobster Raft Grow-out for Northern Australia

Project number: A.3.2021116

Progress report: 2023/24 Q3

Date: 14 April 2024

This project is funded by the CRC for Developing Northern Australia (CRCNA), as part of the Australian Government's CRC Program, with additional support from the Fisheries Research and Development Corporation (FRDC).

WP1: Farm site environment

Design, implement and refine environmental monitoring and management plan (EMMP) for onshore lobster grow-out; 01/07/2022 – 15/04/2024.

General objective

Examine the impact of onshore raft production on water quality and the effect of the environment on pond productivity. Over 2 years (2 stocking events and 1 full production cycle, pilot rafts and lagoon reference site).

Methodology

Since the fourth quarter of 2022/23, water quality monitoring has been conducted employing two distinct methodologies. The conventional approach entails manual recording of morning and afternoon water quality parameters utilising a handheld probe, which adheres to standard procedures. The second methodology utilises the automatic OxyGuard system, operating autonomously as a continuous monitoring system encompassing the Toomulla Beach site, ensuring 24-hour surveillance. Data retrieval is centralised on a monitor, with comprehensive accessibility for analysis purposes. Presently, both methodologies are concurrently employed for water quality assessment.

The conventional method employed for assessing water quality involved the regular recording of water quality parameters twice daily using a handheld probe at two specific locations within the pond: the side of the raft nearest to the paddlewheel and the extremity furthest from it. Daily evaluations included measuring dissolved oxygen (DO), pH, temperature, salinity, and rainfall. Furthermore, bacterial counts on agar plates, nutrient levels and microalgae indicator counts were performed once a week. It is noteworthy that continuous water quality monitoring is ongoing, and the data presented in this report encompasses observations from the years 2023 and 2024.

Summary

Between December 2022 and April 2024, a cumulative total of 886 juvenile lobsters were introduced into the onshore raft system at the Ornatas Toomulla site. Throughout the project duration, environmental conditions mirrored those of North Queensland's tropical climate, characterised by significant variations between the summer and winter seasons. Summer conditions, typically marked by strong and frequent rainfall events, resulted in low salinity and high water temperatures, whereas dry winter conditions led to high salinity and low temperatures. Each scenario affected overall water quality, causing conditions to fluctuate frequently outside the known optimal range for Tropical Rock Lobster (TRL) aquaculture. Nitrogen compounds (ammonia, nitrite, and nitrate) as well as phosphate levels remained within the acceptable range for marine organisms for most of the monitoring period. The minimal release of nutrients by lobsters and the gradual decomposition of uneaten feed over time suggest a limited impact of lobster biomass on the system's water quality, with no necessary management actions required. The trends in water quality parameters are discussed below. Overall, the ambient window of optimal water quality for Tropical Rock Lobster growth is limited in exposed onshore systems, primarily due to high temperature in the wet season and high salinity in the dry season, which require management of water quality parameters in grow-out systems. Further information is required about 'safe' water quality ranges for lobster aquaculture.

Rainfall

According to the Rollingstone meteorological station (32098), the cumulative precipitation for the area was 1564.2 mm in 2023 and 1333.9 mm from January to the 9th of April 2024. In 2023, January to April represented 82.2% of the total annual rainfall; from now on, these months will be referred to as the heavy rain period. Figure 1 displays the daily rainfall recorded at Toomulla at the Ornatas aquaculture site and the Rollingstone meteorological station.

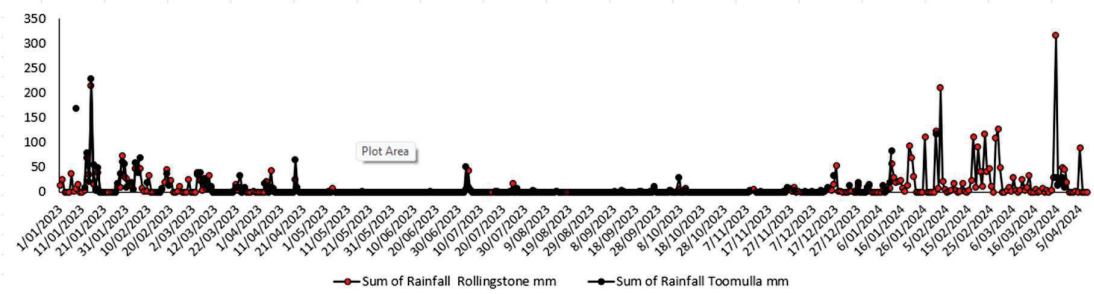


Figure 1. Total monthly rainfall recorded at Toomulla and Rollingstone's station as sourced from the Bureau of Meteorology's website

Temperature

Figure 2 depicts temporal variation in water temperature within the grow-out system throughout 2023 and 2024 (Jan – Apr). During the heavy rain period, the water temperature peaked at 34.9°C in January and dropped to a minimum of 25.9°C in April. Temperatures exceeding 30°C were observed throughout this period, while from May to October, the maximum water temperature recorded was 28.8°C. The lowest temperatures recorded in May and June were 21.7°C, with the coldest temperature of the year occurring in late July at 20.8°C."

The optimal temperature for the growth of wild-caught Tropical Rock Lobster, *Panulirus ornatus,* has been documented at 27°C, falling within an acceptable range of 25 to 31°C, as reported by Jones (2009). Uy et al. (2023) conducted research on this species, reared from hatch, and found that the specific growth rate peaked at 28°C but declined at 32°C. Notably, the temperature range conducive to the highest growth rate for Tropical Rock Lobsters (TRL), approximately between 27 and 28°C, was identified 10.9% of the time at the Toomulla Beach site during 2023. Since temperature is the most challenging factor to control when the body of water is fully exposed to natural fluctuations, the safest months to hold TRL for optimal growth in the grow-out facility during 2023 were September, October, and November, when the water temperature averaged 25.52 ± 1.07°C, 26.43 ± 1.07°C, and 27.35 ± 1.35°C, respectively. However, during those months, the lowest recorded temperature was approximately 24°C. In 2024, the installation of a cooling tower in late January prevented the water temperature from exceeding 32°C, which had occurred in the previous summer in early 2023.

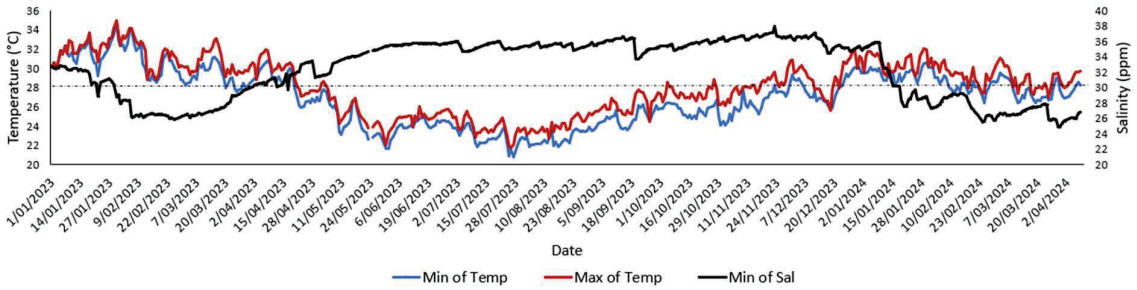


Figure 2. Daily average variation of temperature (°C) and salinity (ppt) in the onshore raft system during 2023 and early 2024. The dashed horizontal line indicates the optimal temperature of 28˚C.

Salinity

Salinity fluctuated between summer and winter during 2023 and 2004 (Figure 2). Summer rainfall events led to a decrease in salinity, while the dry winter period resulted in an increase in this parameter. The lowest salinity levels of 25.9 and 24.89 ppt were recorded in February 2023 and March 2024, respectively. During the dry winter conditions, salinity peaked at 38.1 ppt in November 2023 (Figure 2). Research on salinity tolerance in Tropical Rock Lobsters is limited. Jones (2009) conducted a study revealing that a salinity level of 35 ppt was optimal for growth performance in a 91-day experiment, outperforming conditions of 30, 25, and 20 ppt. Tolerance to hyposaline conditions was observed at 25 ppt, but low growth rates were recorded at 20 ppt. It is noteworthy that cannibalism might have influenced the enhanced growth observed at 35 ppt. Additionally, short-term experiments lasting 48 hours, conducted by Spencer et al. (2023), indicated that *P. ornatus* can tolerate salinity levels ranging from 20 to 40 ppt.

Notably, salinity levels exceeding 30 ppt were documented 85.7% of the time within the Toomulla grow-out system throughout 2023. However, in the period from February to April 2024, salinities below 30 ppt were recorded. A salinity range between 25 and 35 ppt was documented 45.4% of the time, notably during the period from January to April 2023, and in February and April 2024 (with January 2024 recording salinity levels over 35 ppt and March recording levels below 25 ppt). It is crucial to highlight that the introduction of freshwater since August 8th, 2023, reduced salinity to tolerable levels for TRL grow-out.

Alkalinity and pH

According to oceanic water quality standards, the recommended optimal level of alkalinity for TRL is suggested to be in the range of 2.5-3.2 mEq/L (indicated by red doted lines in Figure 3). However, a study conducted on *Panulirus homarus*, where survival and growth were assessed at alkalinity levels of 1.36, 2.5, 4.0, and 5.5 mEq/L, found that the highest survival rate occurred at 4.0 mEq/L (86.7%). This survival rate was significantly higher compared to that at 2.5 mEq/L (64.4%) and 1.36 mEq/L (60.0%; Middlemiss et al., 2016)

In 2023, the range of alkalinity in the grow-out system was between 1.9 and 3.2 mEq/L (Figure 3), while in 2024 the range was between 2.4 and 3.2 Meq/L. The lower alkalinity levels observed in the initial months of 2023 were attributed to substantial rainfall, resulting in a dilution of ions that impacted the buffering capacity of seawater in the grow-out system. Additionally, no addition of chemicals to adjust water quality in early 2023 also led to alkalinity not reaching detection level (≤ 1.9 mEq/L). Throughout the remainder of the year, fluctuations in alkalinity were influenced by several factors, including the introduction of sodium bicarbonate and dolomite since March 2023 to elevate Calcium and Magnesium levels, pH, and alkalinity. Water exchange from the reservoir, rainfall events, and freshwater additions to the system aimed at preventing salinity from approaching levels close to 40 ppt during winter.

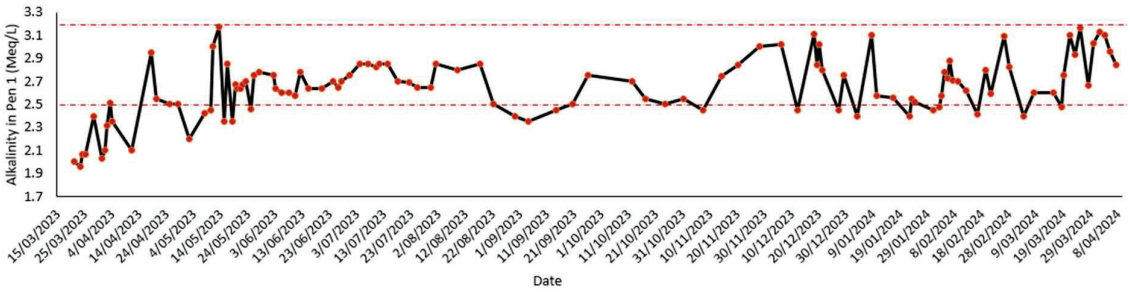


Figure 3. Weekly alkalinity variation in the onshore raft system. Values of 1.9 Meq/L indicate below detection level. Minimum accepted level 2.5 Meq/L and maximum level 3.2 Meq/L.

In accordance with oceanic water quality conditions, the recommended optimal pH range is 8.1-8.3. Similar to alkalinity, the pronounced pH fluctuations observed during the initial months of the year can be attributed to heavy rain events characteristic of the summer season (Figure 4). The higher pH levels maintained throughout the rest of the year are linked to the introduction of calcium and magnesium compounds into the system, implemented since March 2023. Elevated pH levels in August and September, surpassing the suggested range, are attributed to a lack of water exchange from the reservoir (as the reservoir was also out of range) and the addition of calcium carbonate. Conversely, pH levels below 8.2 at the end of the year are associated with rainfall events, reflecting the acid pH of rainwater.

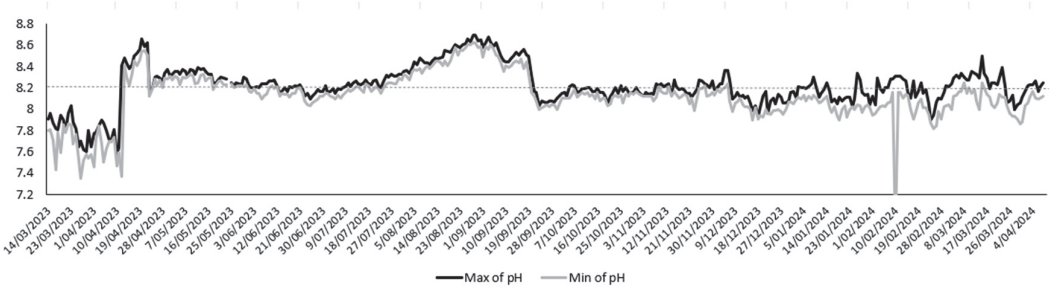


Figure 4. Daily average variation of pH in the onshore raft system at Ornatas Toomulla Aquaculture Facility.

Oxygen saturation

In the initial months of 2023 and 2024, pronounced fluctuations in dissolved oxygen levels were linked to heavy rainfall. The substantial rain volume resulted in a rapid decline in salinity in both the reservoir and the new grow-out system. During this period, the use of mechanical aeration was avoided to prevent the mixing of fresh rainwater and marine water in the pond, thereby averting further reductions in salinity. In addition to the lack of mechanical aeration the heightened biological oxygen demand by heterotrophic bacteria contributed to a further reduction in dissolved oxygen (DO) (Buike, 2018).

Following the conclusion of the early-year rainy seasons, no extreme declines in oxygen saturation were observed. Average oxygen saturation displayed typical diurnal fluctuations, with lower levels in the morning attributed to oxygen consumption by microalgae and lobsters, coupled with CO2 production, during the night.

As the drier season progressed from the end of March in 2023, salinity steadily increased. Unlike earlier in the year, mechanical aeration was consistently sustained during rainfall events to promote the mixing of rainwater with the highly saline pond water. This mixing strategy and continuous aeration played a vital role in sustaining adequate levels of dissolved oxygen (DO), as illustrated in Figure 4.

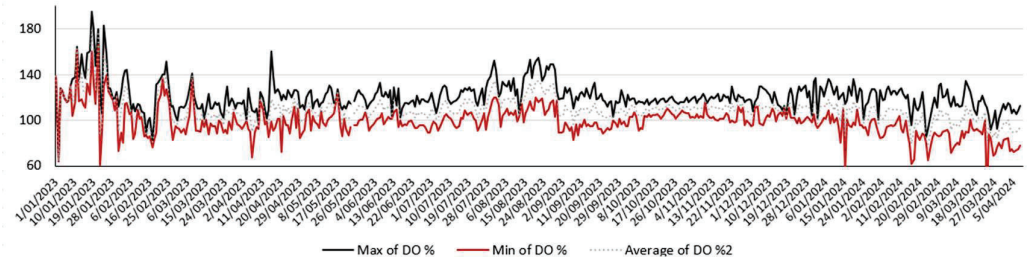


Figure 4. Daily average variation of dissolved oxygen (DO) in onshore lobster enclosures.

Bacterial counts

Similar to the variation observed in oxygen levels, bacterial numbers in the grow-out system exhibited reduced fluctuation after the conclusion of the rainy season in the initial months of 2023 (Figure 5). The number of heterotrophic colony-forming units (CFU) dropped below the 1000s per 100 µL since May and the lower levels were maintained throughout the rest of the year. These bacterial colony counts can be used to set limit levels that prompt management actions, such as the addition of probiotics or water exchange. For instance, from May to December 2023, the average CFU count was 417 ± 224 /100 µL, and no abnormal juvenile mortalities were detected. This establishes a range of 200 to 600 CFU/100 µL as a baseline (normal), where no management action is required.

Similarly, after the conclusion of the rainy season, the number of bacterial colonies growing on TCBS (thiosulfate-citrate-bile salts-sucrose), presumptively of the *Vibrio* genus, did not surpass those growing in the generalist medium (heterotrophic) as indicated in Figure 5. Since a higher relative abundance of *Vibrio* may suggest a potential risk to TRL due to toxins that could lead to diseases in lobsters and other crustaceans, a low *Vibrio* to heterotrophic ratio

can potentially serve as another health baseline indicator. Notably, in 2023, only 3.5% of the sampling times showed a ratio above 1 (Figure 6).

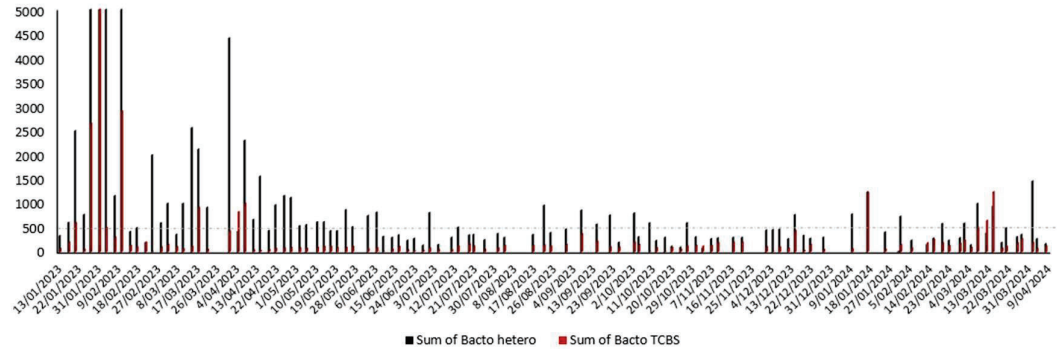


Figure 5. Weekly variation in abundance measured in colony-forming units (CFU/ 100 µL) of culturable heterotrophic bacteria (black bars) and presumptive *Vibrio* sp. (red bars) in the onshore raft system.

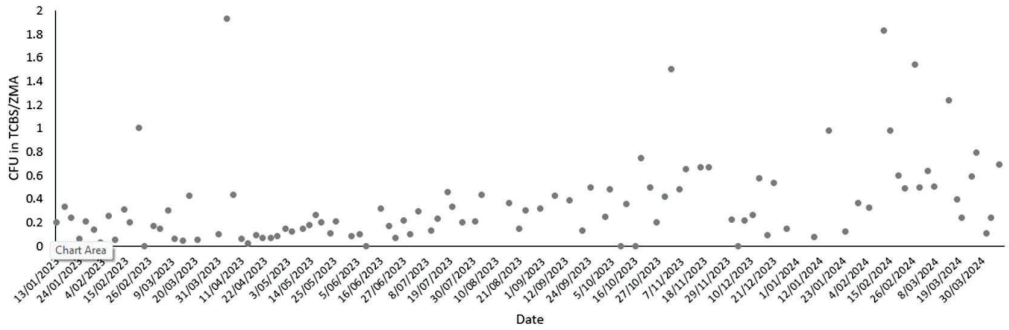


Figure 6. Weekly variation in the ratio of heterotrophic to presumptive *Vibrio* CFU in the onshore raft system.

Water quality management

In summary, there was significant rainfall in the initial months of 2023, which had a multifaceted impact on water quality, resulting in decreases in pH, dissolved oxygen (DO), and salinity. This influx of freshwater also led to an increase in heterotrophic bacteria. Additionally, the diluting effect of rainwater contributed to a reduction in alkalinity. To mitigate the impact of freshwater intrusion, operational adjustments were made. The paddle wheel, typically used for aeration, was turned off to prevent water mixing. Instead, higher salinity water from the reservoir was selectively pumped into the bottom of the pond, displacing surface freshwater due to its lower density. Subsequently, the pond was drained from the top surface of the water column where freshwater had accumulated. This management strategy ceased when the salinity in the reservoir was compromised, by a lower salinity level due to rainfall compared to that in the
pond.

To enhance pH, alkalinity, Ca and Mg levels in the grow-out system, dolomite, sodium bicarbonate and calcium chloride were introduced from the end of March (Table 1; Figures 7 and 8). As illustrated in Figures 7 and 8, there was a notable and rapid increase in Ca and Mg levels, respectively, early in April 2023. Figures 3 and 4 demonstrate a similar pattern for alkalinity and pH, respectively. To prevent bacterial microbiome imbalance and potential diseases, a commercial probiotic (MicroPlus) was employed. This probiotic combines several bacterial strains to promote aquatic bioremediation—assimilating waste and demonstrating antagonistic properties to inhibit growth of opportunistic bacteria such as *Vibrio* spp. While not subjected to *in vitro* testing or to a field experiment, the introduction of this probiotic in April 2023 may have played a role in

contributing to the stability of the bacterial community within the system, potentially indicated by a reduced variation in colony-forming units (CFU) observed in media plates over time.

Table 1. Total amount of sodium bicarbonate, dolomite, and probiotic added to the grow-out raft system.

	Fresh water (L)	Na Bicarbonate (Kg)	Dolomite (Kg)	MicroPlus (mL)	Calcium chloride (Kg)
2023	**412500**	**740.2**	**145**	**1300**	
2024	**110**	**447.5**	**227.5**	**600**	**260**
Jan	110	72.5	32.5	200	10
Feb		125	110	200	100
Mar		250	50	200	125
Apr			35		25
Grand Total	**412610**	**1187.7**	**372.5**	**1900**	**260**

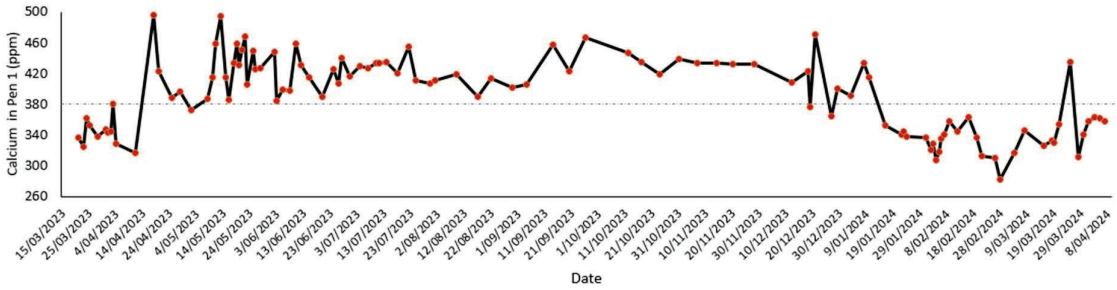


Figure 7. Weekly variation in calcium concentration in the onshore raft system. Grey dotted line indicates the lower limit value within the optimal range (based on ocean water quality).

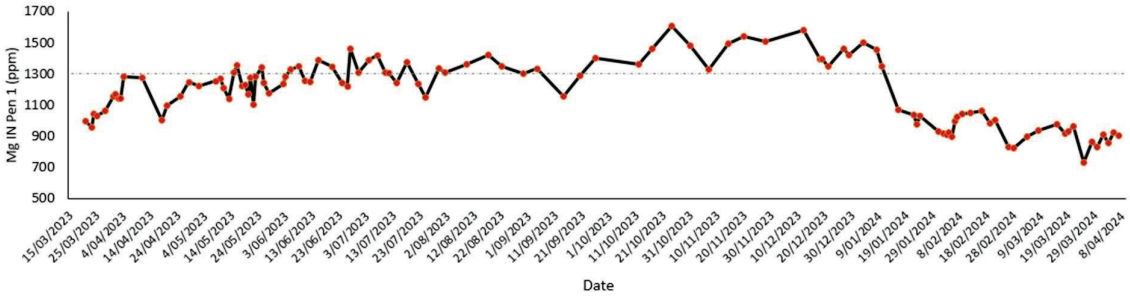


Figure 8. Weekly variation in magnesium concentration in the onshore raft system. Grey dotted line indicates the lower limit value within the optimal range (based on ocean water quality).

Due to the dry season resulting in reduced rainfall, salinity in both the reservoir and the grow-out system reached optimal levels for growth, as indicated by previous studies (35 ppt). However, the limited rainfall from May onwards led to a gradual increase in salinity above 35 ppt (Figure 2). To maintain salinity within acceptable levels, a total of 75,000 L of freshwater was added to the system in August, followed by another 75,000 L in September, 25,000 L in October, 125,000 L in November, and 112,500 L in December (Table 1). This influx of freshwater contributed to fluctuations in alkalinity (Figure 3), which were addressed through the gradual addition of sodium bicarbonate.

During the previous quarter (Jul – Sept 2023), the rise in salinity in the reservoir limited water exchange in the grow-out system, and salinity was primarily controlled by the addition of freshwater. The introduction of sodium bicarbonate to the onshore system, coupled with the absence of water exchange, resulted in an increase in pH above the recommended levels.

Current and future plans

As previously highlighted, salinity and temperature pose significant management challenges for the grow-out of Tropical Rock Lobsters in seasonal tropical environmental conditions. The utilisation of seven reservoirs (Figure 9) served as a strategy to secure saltwater, as evaporation increased salinity while the volume of water decreases over time. However, due to low levels of rainfall since May 2023, freshwater was purchased to adjust salinity across site. In the long term, there are plans to shift from creek water to ocean water delivery for the seawater supply with the intent to deliver higher quality water year-round. Further research is required to define 'safe' water quality ranges for lobster aquaculture, where high survival and potentially lower growth can be maintained to secure land-based production.

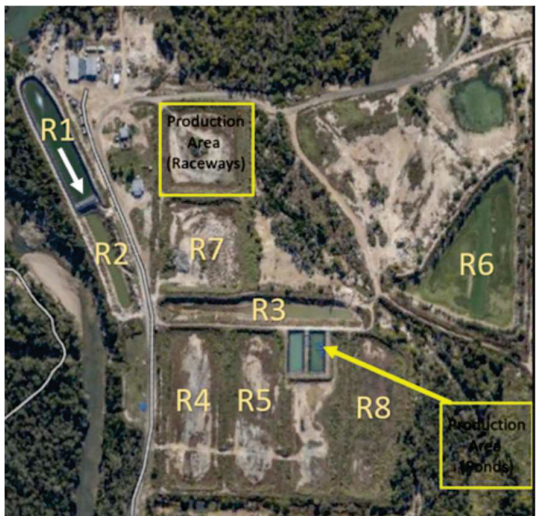
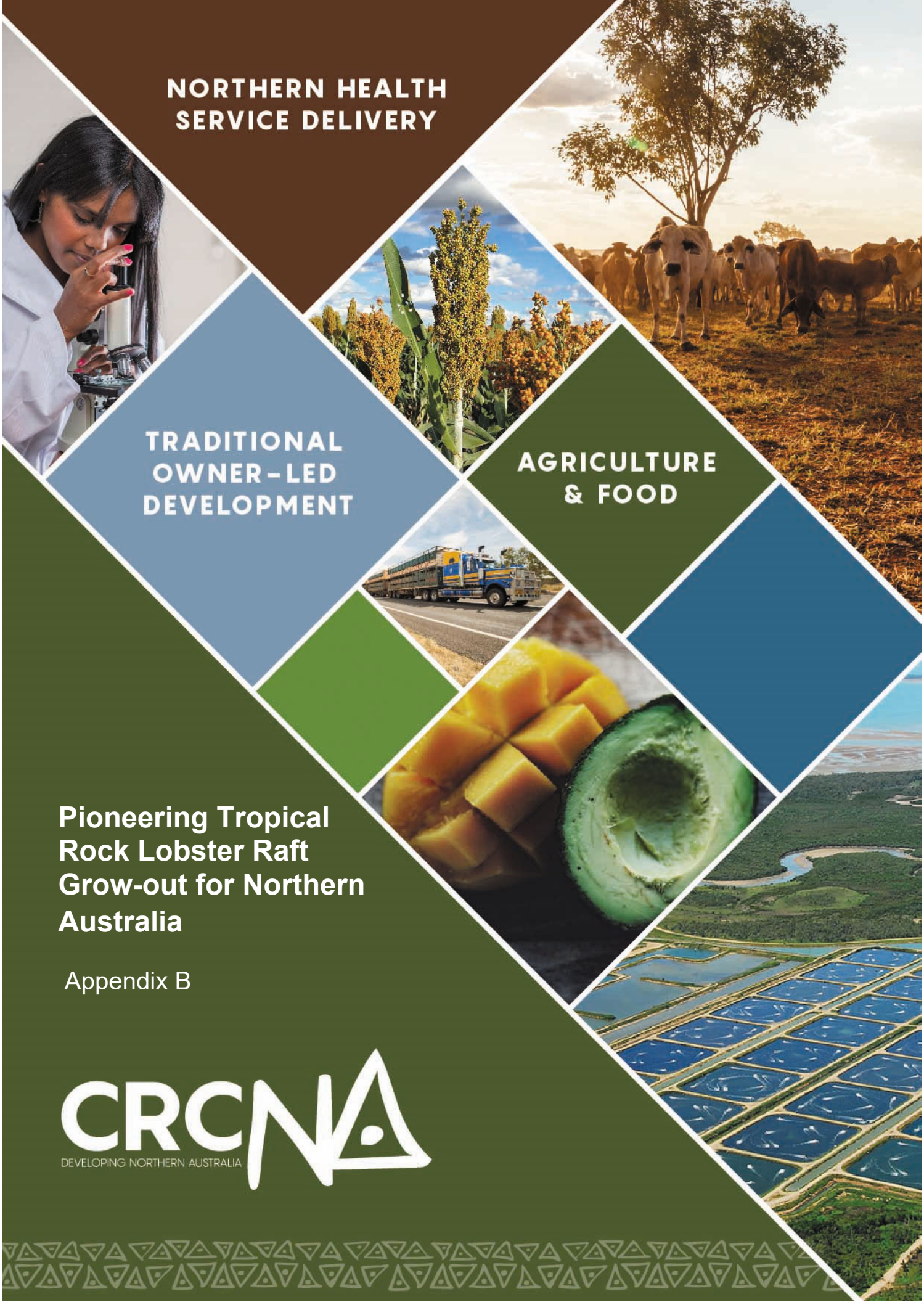


Figure 9. Aerial photography of storage reservoirs and channels (R#)indicating the location of the onshore grow-out system currently operating.

References

- Buike, P. (2018). Rainy season effects on shrimp grow-out ponds. Global Seafood Alliance Advocate. Retrieved from https://www.globalseafood.org/advocate/rainy-season-effects-on-shrimp-grow-out-ponds/
- Jones, C. M. (2009). Temperature and salinity tolerances of the tropical spiny lobster, Panulirus ornatus. Journal of the World Aquaculture Society, 40, 744-752. https://doi.org/10.1111/j.1749-7345.2009.00294.
- Middlemiss, K., Urbina, M.A. and Wilson, R. W. (2016). Effects of seawater alkalinity on calcium and acid base regulation in juvenile European lobster (*Homarus gammarus*) during a moult cycle. Comparative Biochemistry and Physiology - Part A: Molecular and Integrative Physiology, 193, 22-28.
- Spencer, E.L., Fitzgibbon, Q.P., Day, R.D., Trotter, A.J. and Smith, G.G. (2023). Effects of acute salinity stress on the survival and haemolymph biochemistry of juvenile tropical rock lobster, *Panulirus ornatus*, at different moult stages. Aquaculture, 573: 739597.



Pioneering Tropical Rock Lobster Raft Grow-out for Northern Australia

Appendix B

CRCNA
DEVELOPING NORTHERN AUSTRALIA

ornatas

Innovate to grow

Securing the Future of Aquaculture

Grow-out Best Practice

Date:	**2/04/2024**
Batch code:	**Across all**

Grow-out Trial Pond 1 and 2

This document describes the operating specifications and water quality parameters for the grow-out of puerulus and juvenile stages of the Tropical Rock Lobster *Panulirus ornatus*

Figure 1. Aquatec floating devices fully assembled in the Ornatas' grow-out system.

Operating conditions:

	Parameter	Conditions
Vessel	Aquatec Raft. Floating device and large enclosures (Figure 2).	Follow the *Lobster cage with submersible cage system Manual Book* for instructions on floating device and enclosure assembly. During enclosure assembly ensure the access gate is correctly attached to the frame avoiding holes >10mm (Figure 3).
Operating specifications	Volume/Flow Rate/Velocity	Total production volume is 146,552 L (16 enclosures). 2x Badu Speck Pumps and PVC/POLY manifold to provide flow of water into each pen (Figures 4 and 5).
	Enclosure surface area and volume:	Bottom Floor (double layer mesh): 6.75m2 Volume: 9180L
	Stocking density	Under investigation: potential of 60 harvest size (1.2kg) animals per cage.
	Hides	Locate a minimum of 3 shelf hides on the opposite walls to the feeder. Use PVC pipe to keep the hide at approximately 40cm to the bottom. Attach the hide to the hinge of the enclosure wall using cable ties. Attach ropes to lift the hides and facilitate observation and cleaning (Figure 6). Hides are kept opposite to the feed area to separate the feeding and the moulting animals.
	Photoperiod	Natural day night cycle
	Aeration	One eight-impeller paddlewheel aerator is used per grow-out system (800,000 L of water). Depending on salinity levels use the auto setting during winter (dry period) and manual setting during summer. The paddlewheel must be turned off to avoid mixing in rainwater if salinity is ≤ 30 ppt (Figure 1). As a backup method of aeration, a venturi is plumbed inline in the turnover manifold line.
Data collection	Weight	During full biometrics (seasonal) and grading (as needed).
	Length (CL, TL)	During full biometrics (seasonal) and grading (as needed).
	Condition	During full biometrics (seasonal): appendages missing, eye health, sex, abdomen colour,

		biofouling (scale 1-5), tail fan necrosis (scale 1-5).
	Daily	Water Quality (inlet and outlet sides): Dissolved Oxygen, Temp, pH, Salinity.
	Weekly	Total number of lobsters per enclosure (counts). Feed consumption WaterLink® Spin Touch®: Nitrite, Nitrate, Ammonia, Phosphate, Calcium, Magnesium, pH and Alkalinity. Algae samples. Bacterial samples. Water quality management: sodium bicarbonate, calcium chloride, magnesium chloride, dolomite, Probiotic (Micro Plus; Pure Aquatics), salt and fresh water.
Feeding management	Feeder type	12-hour Belt Feeders and feed tubes (Figure 7). Arvo Tec Feeder. Under trial for upscaling (Figure 8)
	Feed type:	JF5 formulation (UTas-Ornatas IP - patented)
	Feed times	12 Hour Belt Feeders: 3 feeds spread out evenly over the belt. Reset belt feeders no earlier than 1pm each day.
	Feed ration	Initial ration based on 2% of the biomass in each enclosure. Increased as needed based on feed consumption observation and new biomass calculated and updated weekly.
	Cleaning	Weekly when pens are lifted for counts. Excess feed and small moult fragments are removed.
Grading and duration	Grading frequency	In conjunction with biometrics, normally at the start and end of each season: winter/dry and summer/wet.
	Duration	Attempt to conduct grading every 3 months, aligning with seasonal changes. Note: schedule may vary depending on water quality conditions.

Water Quality Parameters:

Parameter	**Range**
Dissolved Oxygen (%)	80%-120%
Dissolved Oxygen (mg/L)	@28°C 6mg/L-9mg/L

Salinity (ppt)	25ppt-35ppt
Temperature (°C)	25-30°C
pH	8.15-8.3
Calcium (ppm)	380-480ppm
Magnesium (ppm)	1300-1500pm
Alkalinity (Meq/L)	2.6-3.3 Meq/L
Ammonia NH4 (ppm)	<1ppm
Unionized Ammonia NH3 (ppm)	<.25ppm
Nitrite NO2 (ppm)	<5ppm
Nitrate NO3 (ppm)	<100ppm
Phosphate (ppm)	TBD

a.

b.

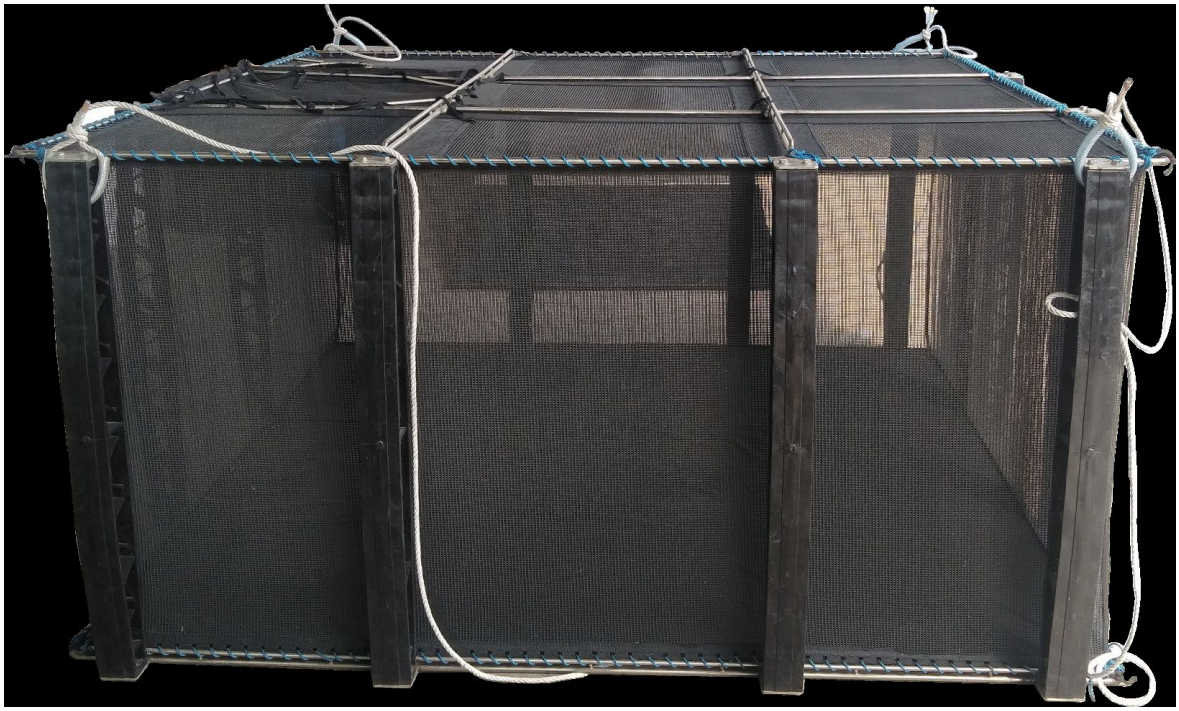


Figure 2. Assembled Aquatec raft floating device (a) and large enclosure (b) used for TRL grow-out.

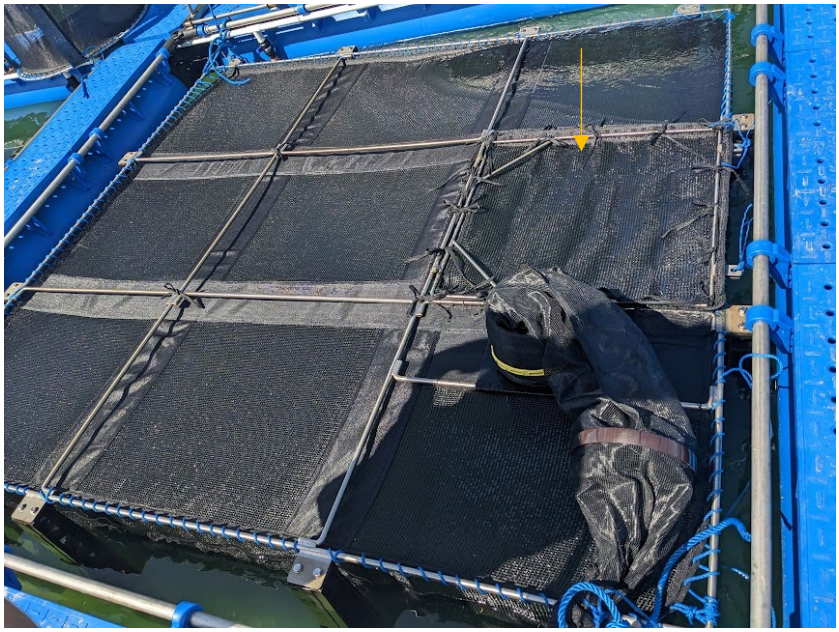


Figure 3. Large enclosure used for TRL grow-out. Upon assembly, ensure that the access gate, indicated by the yellow arrow, does not have holes larger than 10 mm.

Figure 4. Infrastructure connected to two pumps required to support water flow in enclosures used for TRL grow-out.

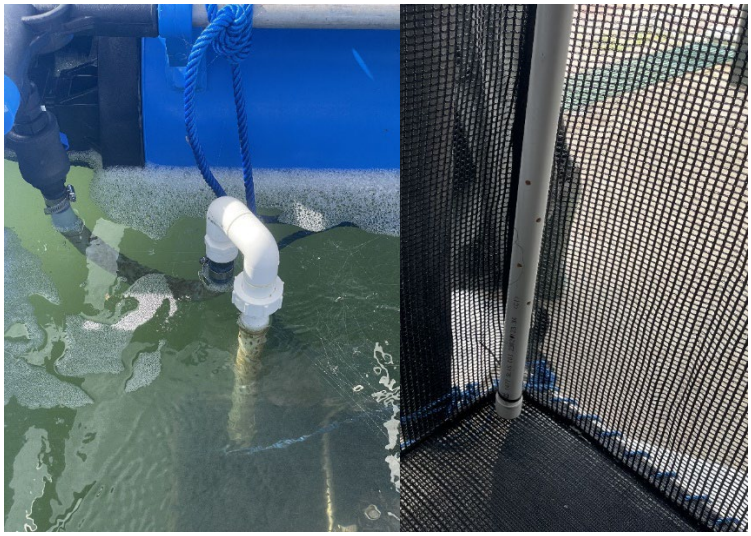


Figure 5. PVC/POLY manifold assembly to ensure water flow in enclosures used for TRL grow-out.

Figure 6. Shelf hides installed in a large enclosure ready to be attached to the floating device of the raft.

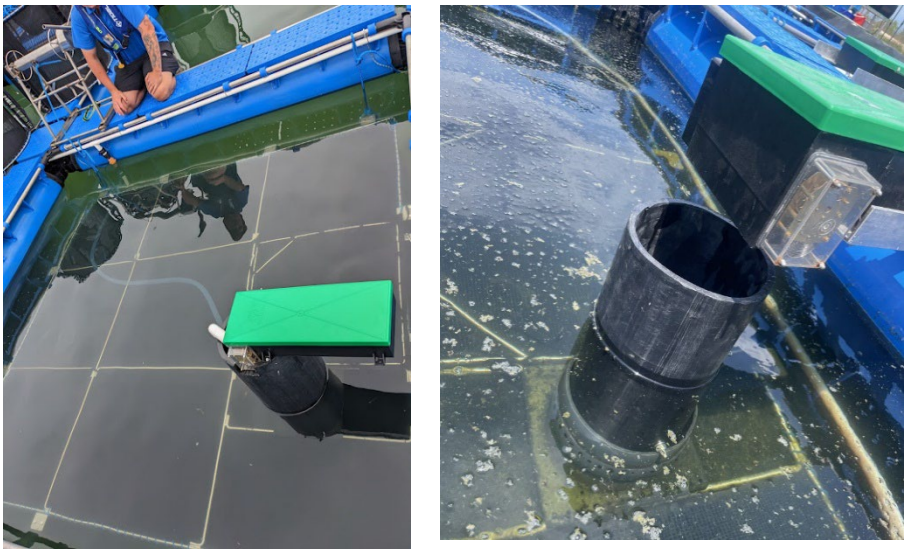
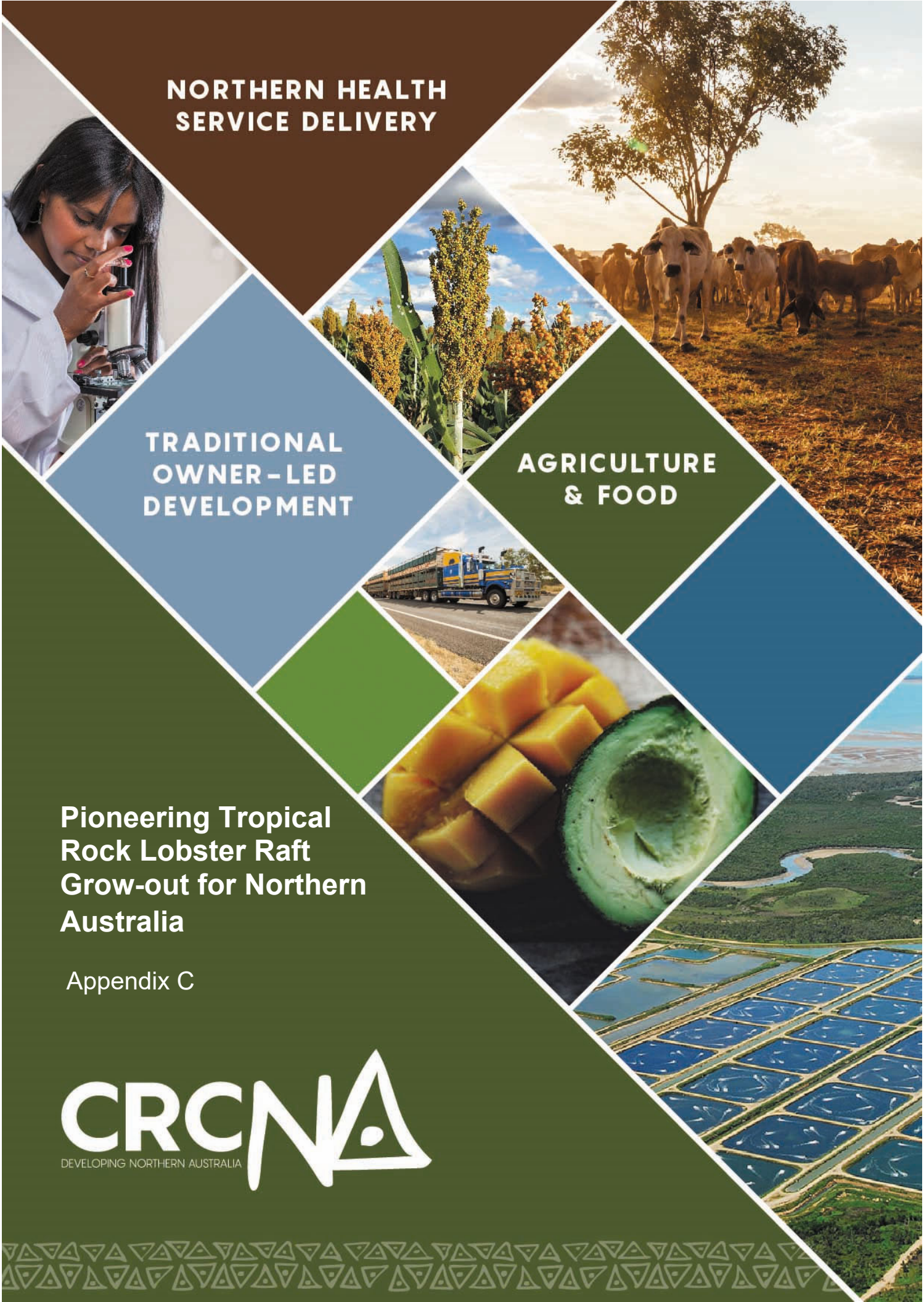


Figure 7. Bel feeder installed on a large enclosure (left). Close-up of a feed tube and the clock system of a belt feeder (right), which activates the belt to automatically provide three feed rations during a period of 12 hours.

Figure 8. Arvo-Tech feeder installed on a large enclosure. Feeder undergoing testing.



Pioneering Tropical Rock Lobster Raft Grow-out for Northern Australia

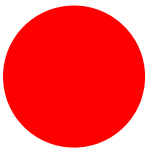
Appendix C

CRCNA
DEVELOPING NORTHERN AUSTRALIA

Section:	Growout	Document No:	1	Version:	1
Prepared by:	BS	Date:	03/04/24	Supersedes:	
Checked by:		Date:		Date Issued:	
Approved by:		Date:		Review Date:	

Operations On and Around Growout Trial Ponds

Relevant Biosecurity Zones:



IMPORTANT: This SOP is relevant to *Red* biosecurity zones, adhere to the protocols for this zone

(Please remove and select appropriate colour:)

- *Red – Broodstock & Quarantine, Artemia Production, Primary Filtration, Pond Grow-out, Waste & Discharge Channel, Bacteriology & Disease Management*
- *Amber- Juvenile Production*
- *Yellow – Feed manufacturing, Clean water tank farm, Larval Rearing*
- *Green – Administration, Lunch & Amenities, Workshop, Accommodation*

Report all incidents immediately. Complete a Take 5 prior to work. If additional risks (Not covered in this SOP) are identified, complete a separate risk assessment form located in the lunchroom. DO NOT PARTICIPATE IN / CARRY OUT THE ACTIVITY IF YOU ARE NOT PHYSICALLY / PSYCHOLOGICALLY CAPABLE. Discuss any fitness for work requirements (e.g. fatigue, injury, illness & medication effects) with your Line Manager.

Scope & Responsibility: (detail who, what and when this SOP applies)

To all employees working on and around the Growout ponds.

Risks: (List the risks that are associated with this activity / plant /equipment)

- Slips, trips, falls
- Cuts with equipment or during animal handling
- Drowning
- Musculoskeletal injuries (muscles, joints and bones) such as sprains, muscle tears and strains due to overexertion, improper lifting techniques, or lifting loads beyond the operator's capacity
- Exposure to hazardous chemicals that can lead to injuries
- Heat related illnesses (heat exhaustion, heat stroke, heat cramps, and heat rash), dehydration and sunburn
- Electrocution from electrical equipment near water
- Wildlife interaction (crocodile, snake, spider, lizards, birds)

Section:		Document No:	1	Version:	1

PPE:

Hazards:

SDS's:

Calcium Chloride 74% Flake_SDS_1Jan2022.pdf
Dolomite-GHS-2021.pdf
Salt_sds 14_02_2022.docx
Sodium Bicarb SDS.pdf
Magnesium Chloride.pdf
Micro+1000 SDS[3952].pdf
Ethanol (Industrial Methylated Spirit)_SDS_19Dec2020.pdf
SURF LIFE SAVING AUSTRALIA DAILY MARINE FRIENDLY SPF50+.pdf

Purpose:

To safely work on and around the Growout Ponds during daily and weekly routines.

Frequency:

Daily/Weekly

Equipment Required: (Not including PPE – identified above, including first aid)

- Daily check list (Figure 1)
- Primary Water and Growout ProDSS Water Quality Probe (Figure 2)
- Gloves
- Nets
- Buckets
- Polarized Sunglasses
- Sample Jars

Procedure:

- Always prepare for sun exposure when working on the rafts. Sunscreen, sunglasses and broad brimmed hat is recommended.
- When approaching the ponds in any vehicle you must park parallel with the pond walls. Leaving the vehicle in gear and with hand break on.
- Follow the morning and the afternoon daily check list: New pond Checksheet.xlsx

- An observation walk must be undertaken at the start of the day's routine around the pond walls and rafts to ensure everything is in good working order e.g. No landslides, pens tied up properly, plumbing intact, no potentially harmful wildlife, and no signs of escaped lobsters
- Water quality must be taken at the front and back of the raft, using the ProDSS drop the probe down 1m and wait for parameters to settle before recording (usually 30-40 seconds for the first reading after turning on the unit). All parameters recorded here: Daily Data Capture 2023_24.xlsx
- Assess for the presence of a halocline, bring the probe to the surface and observe the reading within the top 30cm of water (no need to record)
- Check that the cooling towers are running correctly (Ponds SOP #4 – Cooling Tower Operation)
- Oxyguard probes are to be cleaned and serviced (Ponds SOP #5 – Oxyguard Pond Maintenance)
- If halocline is not present, paddlewheels should be switched to AUTO. This setting will turn the paddlewheel off when it rains.
- Before starting any exchange into the ponds, the filter must be backwashed until it runs clear (Ponds SOP #2 – Filter and Exchange Processes).
- To ensure safe lifting of pens for animal counts, cleaning, and grading, a minimum of two people must be present to operate and move the pen winch (Ponds SOP #3 – Winch and Pen Lifting) (Ponds SOP #9 – Grading/Biometrics and Animal Handling.
- Belt feeders are to be cleaned with ethanol weekly to prevent build-up of mould.
- Spin touch and Bacto samples are to be taken using the sample pole from 1m down in the water column (Ponds SOP #6 – Water Quality Samples).
- Algae samples are taken with the sample pole according to Ponds SOP #7 – Algae Sample, Identification and Counts.
- Feed stocktake is to be undertaken weekly, comparing the amount of feed in stock to the weekly outgoing usage. This assessment helps determine the remaining weeks' worth of food and informs the Feed Team about the appropriate size of the order needed." Link to the Stocktake Feed Calculation tool: Daily Data Capture 2023_24.xlsx
- Place a feed order when required. Link to the form: Grow out feed order sheet.xlsx
- Follow the best practice document for resetting belt feeders. Link to document:: Best Practice Growout V1 2.4.24.docx
- Assess the likelihood of rain for the night. If uncertain, it is recommended to turn off the paddlewheels for the night. When paddlewheels are turned off overnight for consecutive days (2 or more days), monitor OxyGuard oxygen saturation and pH levels overnight (from 6 pm to 6 am) at least twice per week. Should saturation levels fall below 80%, promptly communicate with the Grow-Out Manager.
- Any buffering agents added must adhere to proper safety protocols, utilizing appropriate Personal Protective Equipment (PPE) as outlined in each chemical's Safety Data Sheet (SDS) and Pond Standard Operating Procedure (SOP) #8 – Buffering Agent Additions. Buffering agents include: Mermaid Flossy Salt, Sodium Bicarbonate, Magnesium Chloride, Calcium Chloride, Dolomite, and Probiotic. All quantities added must be recorded in Daily Data Capture 2023_24.xlsx

Figures

Growout Checklist	Date								Comments:
	Day	Mon	Tue	Wed	Thur	Fri	Sat	Sun	
	Technician								
MORNING	Cage Inlet Pump On								
	Pontoon Walkover								
	Pond WQ								
	Check Halocline								
	Cooling Towers Balanced								
	Clean Oxyguard Probes								
	Paddle Wheel ON/OFF/AUTO								
	PW Morning Routine								
	Backwash Filter								
	Pond Inlet On (TIME)								
	Pond Inlet Off (TIME)								
	Morts/Count								
	Excess Feed Removed								
	Feeders Cleaned								
	Cages Scrubbed								
	Spintouch Sample								
	Bacto Sample								
	Algae Sample								
	Feed Stocktake								
AFTERNOON	Cages Secure								
	Cage Inlet Lines Open								
	Pond WQ								
	Reset Belt Feeders/ Fishmates								
	Feed Mussel Shells								
	Add Buffering Agents (amount)								
	Cooling Towers Balanced								
	Paddle Wheel ON/OFF/AUTO								
	All Data Entered								
	PW Arvo and Daily Dis								

Figure 1. Daily checklist to be followed

Figure 2. Primary Water and Growout ProDSS Water Quality Probe

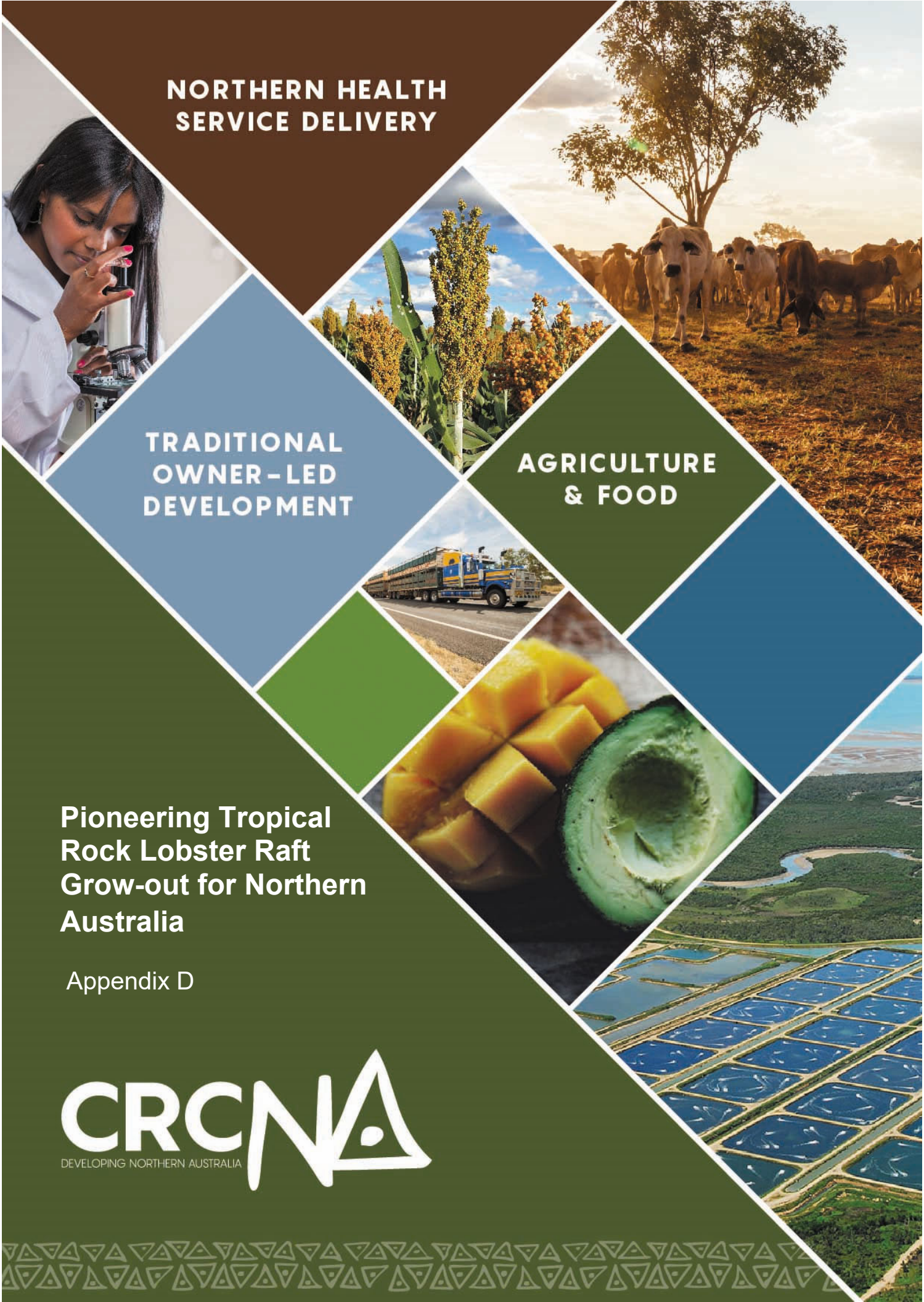
Section:		Document No:	1	Version:	1

Emergencies: (Identify the types of emergency and the emergency response)

- In the event of a fall into the pond, promptly and calmly utilize the rope ladder on the pond wall to facilitate your safe exit. Report as soon as possible to Senior Management. Following this occurrence, it is necessary to complete an incident report once you have dried off and changed into dry clothing.
- Animals deemed dangerous within the ponds must be left undisturbed. Report as soon as possible to Senior Management. Manager to contact licenced/qualified wildlife handlers to facilitate animal removal/relocation.

Related Documentation: (Document resources that can be referenced for further information. Hyperlink if possible)

- New pond Checksheet.xlsx
- Daily Data Capture 2023_24.xlsx
- Grow out feed order sheet.xlsx
- Best Practice Growout V1 2.4.24.docx
- Ponds SOP #2 – Filter and Exchange Processes
- Ponds SOP #3 – Winch and Pen Lifting
- Ponds SOP #4 – Cooling Tower Operation
- Ponds SOP #5 – Oxyguard Pond Maintenance
- Ponds SOP #6 – Water Quality Samples
- Ponds SOP #7 – Algae Sample, identification and Counts.
- Pond SOP #8 – Water quality adjustment
- Ponds SOP #9 – Grading/Biometrics and Animal Handling.



Pioneering Tropical Rock Lobster Raft Grow-out for Northern Australia

Appendix D

CRCNA
DEVELOPING NORTHERN AUSTRALIA

PATHOGEN RISK ANALYSIS FOR AQUACULTURE BIOSECURITY AND TRANSLOCATION OF TROPICAL ROCK LOBSTERS (*Panulirus ornatus*) IN NORTHERN AUSTRALIA

DigsFish Services Report: DF 21-03b
29 November 2021

PATHOGEN RISK ANALYSIS FOR AQUACULTURE BIOSECURITY AND TRANSLOCATION OF TROPICAL ROCK LOBSTERS (*Panulirus ornatus*) IN NORTHERN AUSTRALIA

Prepared by:

Ben Diggles PhD
DigsFish Services Pty Ltd, Banksia Beach, QLD 4507

Prepared for:

SeaRaft Research Pty Ltd,
PO Box 774 Deeragun, QLD 4818

Version Control:

First Draft Hazard ID	27 April 2021
Draft Risk Analysis	20 October 2021
Final Version	29 November 2021

Disclaimer

This document was prepared as an independent pathogen risk assessment. The views expressed are those of the author. DigsFish Services does not warrant that the information in this document is free from errors or omissions. The authors do not accept any form of liability, be it contractual, tortious, or otherwise, for the contents of this document or for any consequences arising from its use or any reliance placed upon it.

Contents

List of Figures

List of Tables

Abbreviations and Acronyms

AAHL	Australian Animal Health Laboratory
AHPND	Acute hepatopancreatic necrosis disease
ALOP	Appropriate level of protection
BMNV	Baculoviral midgut gland necrosis virus
BRMP	Biosecurity Risk Management Plan
CL	Carapace length
cPCR	Conventional PCR
DAWE	Commonwealth Department of Agriculture, Water and Environment
DIV1	Decapod iridescent virus 1
DPIRD	Department of Primary Industries and Regional Development WA
EHP	*Enterocytozoon hepatopenaei*
GAV	Gill associated virus
HgNV	*Homarus gammarus* nudivirus
IHHNV	Infectious hypodermal and haematopoietic necrosis virus
IMAS	University of Tasmania Institute for Marine and Antarctic Studies
IMNV	Infectious myonecrosis virus
LD50	Lethal dose which results in mortalty of 50% of infected hosts
mg/L/min	Total ozone dose (*Ct*) = mg of ozone per L of water per unit time of exposure (min)
MHD	Milky haemolymph disease
MHD-SL	Milky haemolymph disease of spiny lobsters
mJ/cm^2	Microbicidal UV dosage. 1 mJ/cm^2 = 10J $/m^2$= 1,000 $\mu W/cm^2$ per second
MrNV	*Macrobrachium rosenbergii* nodavirus
MSGS	*Monodon* slow growth syndrome
NA	Northern Australia
NACA	Network of Aquaculture Centres in Asia-Pacific
NHP	Necrotising hepatopancreatitis caused by infection with *Hepatobacter penaei*
NT	Northern Territory
OIE	Office International des Epizooties, the world organisation for animal health
ORP	Oxidation/Reduction Potential
PaV1	*Panulirus argus* virus 1
PCR	Polymerase chain reaction
qPCR	Quantitative PCR
QLD	Queensland
RA	Risk analysis
RLO	Rickettsia-like organism
SCAAH	Sub-Committee on Aquatic Animal Health
SMV	Spawner isolated mortality virus
SOPs	Standard operating procedures
TEM	Transmission electron microscopy
TRL	Tropical rock lobster
TRO	Total residual oxidants (from ozone exposure) – measured in mg/L
TSV	Taura syndrome virus
UK	United Kingdom
WA	Western Australia
WSSV	White spot syndrome virus
YHV1	Yellow head virus genotype 1

Non – technical summary

SeaRaft Research Pty Ltd (a wholly owned subsidiary of Ornatas Pty Ltd) have proposed a need for translocation of juvenile cultured tropical rock lobsters (*Panulirus ornatus*) from QLD to WA as part of a research project providing the foundation for the development of a tropical rock lobster (TRL) aquaculture industry in northern Australia. Through consideration of the overall development of a new TRL aquaculture industry in northern Australia, a national group of government biosecurity experts recommended a relatively broad biosecurity risk assessment should be undertaken to examine pathogen risks for a range of TRL life history stages during transfer amongst various domestic jurisdictions. Any movements of tropical rock lobsters between domestic jurisdictions within Australia need to be underpinned by a comprehensive pathogen risk analysis (RA), to inform the development of biosecure translocation protocols before they are likely to be approved by the relevant state jurisdictions. Information generated during the RA can also be incorporated into biosecurity plans and during development of updated biosecurity protocols for Ornatas-operated hatcheries as well as other companies with an interest in lobster aquaculture grow out in northern Australia. The RA can also be used to assist with design of tropical rock lobster disease surveillance programmes, development of diagnostic capabilities, and establishment of other oversight/audit services.

This document presents the results of this risk analysis process. A comprehensive hazard identification process identified at least 39 diseases of potential concern, including 15 viral diseases, 7 bacterial diseases, 3 fungal diseases, 7 protozoan diseases and 4 groups of metazoan disease agents, as well as 3 diseases of non-infectious aetiology. A process of elimination of various insignificant or irrelevant diseases was then undertaken, leaving a priority list of 3 viral diseases, 1 bacterial disease, 1 fungal disease and 3 protozoan diseases that were subject to detailed risk assessment. The 5 specific release pathways examined included introduction of broodstock lobsters into the hatchery in north QLD, intake of water into the hatchery in north QLD, and release of juvenile lobsters into sea rafts for grow out in the waters of northern Australia (QLD, NT, and WA).

Results from the detailed risk assessments found that there was a need for additional risk mitigation for one or more pathways for 7 of the 8 diseases (see summary table below for the outcomes from the risk assessments). These included moderate to high risks of infection with white spot syndrome virus (WSSV), moderate risks of infection with undescribed endemic viruses, moderate to low risks of infection with haplosporidians and *Hematodinium* spp., and low risks of microsporidosis, infection with rickettsia like organisms (RLOs) which can cause milky haemolymph disease, and infection with scuticociliates.

Options for risk mitigation were identified to reduce the risks to within the appropriate level of protection (ALOP). These included protocols for collection of broodstock, testing broodstock for diseases of concern, disinfection of hatchery intake water, identifying optimal biosecurity practices such as use of formulated feeds, separation of cohorts of lobsters during rearing of larvae and juveniles, disinfection of effluent water, testing of larvae and juveniles for diseases of concern, and so on. These options should form the basis of a consultation process that engages Government and stakeholders to evaluate the biosecurity risks involved with the proposed translocations with a view towards identifying practical mitigation options that would reduce the risks identified to an acceptable level. Finally, it should be noted that this risk analysis represents a snapshot of the known disease situation at the time of publication. It will therefore need to be updated on a regular basis in the future as new information on diseases of TRL in Australia becomes available.

Summary table for unmitigated risk estimate outcomes from the risk assessment.

Pathway	Via broodstock into hatchery in north QLD	Via water into hatchery in north QLD	Via juveniles into waters of QLD	Via juveniles into waters of the NT	Via juveniles into waters of WA
Viruses					
Infection with *Panulirus argus* virus 1 (PaV1)	Very low risk 4	Very low risk 4	Very low risk 4	Very low risk 4	Very low risk 4
Infection with undescribed endemic viruses	Moderate risk 12	Moderate risk 12	Moderate risk 12	Moderate risk 12	Moderate risk 12
Infection with white spot syndrome virus (WSSV)	Moderate risk 12	High risk 16	Moderate risk 12	Moderate risk 12	Moderate risk 12
Bacteria					
Milky haemolymph disease of spiny lobsters (MHD-SL) (or similar RLO)	Low risk 8	Low risk 8	Low risk 8	Low risk 8	Low risk 8
Fungi					
Microsporidosis	Very low risk 6	Low risk 9	Low risk 9	Low risk 9	Low risk 9
Protozoa					
Haplosporidosis	Low risk 8	Moderate risk 12	Low risk 8	Low risk 8	Low risk 8
Infection with *Hematodinium* spp.	Low risk 9	Moderate risk 12	Low risk 9	Low risk 9	Low risk 9
Scuticociliate disease	Very low risk 6	Low risk 8	Very low risk 6	Very low risk 6	Very low risk 6

1.0 Introduction

There has been increasing interest for several years now in further development and diversification of the aquaculture industry across northern Australia (NA) (Cobcroft et al. 2020). Recent technical developments in the hatchery rearing of tropical rock lobsters (*Panulirus* spp.) has led to the potential for these to become a high value component of an expanded aquaculture industry in NA in the near future (Hall et al. 2013, Jones 2015). However, it is well known that biosecurity and management of disease risks are very important aspects that need to be considered during the development of any new aquaculture industry, including for lobsters (Langdon 1990, Diggles et al. 2002a, Evans 2003, Stephens et al. 2003, Shields 2011, Behringer et al. 2012a, Stentiford 2012, Ross et al. 2019b).

SeaRaft Research Pty Ltd (a wholly owned subsidiary of Ornatas Pty Ltd) have proposed a need for collection and domestication of wild-caught broodstock tropical rock lobsters (*Panulirus ornatus*) (TRL) from the coastal waters of QLD into a hatchery in QLD, and translocation of juvenile TRL cultured in the QLD hatchery to grow out rafts in Cone Bay, WA as part of research which will underpin the development of a TRL aquaculture industry in NA. Any movements of broodstock or juvenile rock lobsters, especially between domestic jurisdictions within Australia, need to be underpinned by a comprehensive pathogen risk analysis (RA), to inform the development of biosecure translocation protocols before they are likely to be approved by State Government biosecurity authorities. Because of this, DigsFish Services has been engaged to undertake a RA to identify significant disease risks and discuss potential risk mitigation measures that could reduce the biosecurity risks posed by the proposed translocations to within acceptable levels. The RA would also be a useful resource which may help predict the most likely future emerging disease risks for the industry, and could be used to upgrade existing biosecurity management frameworks and operating procedures to effectively mitigate those disease risks alongside the various other risks (e.g. genetic pollution) that may arise during industry development. The information generated during the RA can also be incorporated into existing biosecurity plans through a process of continuous improvement during development of biosecurity protocols for Ornatas operated hatcheries and lobster aquaculture grow out facilities in NA, and any associated oversight/audit services.

This RA was undertaken firstly to: 1. identify diseases which may affect tropical rock lobsters; 2. identify potential pathogens (hazards) which could be translocated between various jurisdictions within NA; 3. identify in a qualitative manner the translocation risk for each hazard of concern, and 4. outline a range of risk mitigation options which could be approved by relevant State biosecurity authorities then implemented in translocation protocols by Ornatas to reduce these risks to an acceptable level. This RA was done using a standardised risk analysis process utilising transparent, science-based decision making, as explained by Diggles and Arthur (2010) and based on international guidelines (OIE 2021a) whilst recognising the relative paucity of information available on rock lobster diseases both internationally and particularly within Australia. Following a comprehensive hazard identification process, each hazard of concern was subjected to risk assessment based on a qualitative assessment of the various risks involved with introduction (release), establishment (exposure) and spread (consequences) of each hazard as assessed using internationally recognised risk analysis methodologies (Diggles and Arthur 2010, OIE 2021a, Diggles 2011, 2017b, 2020b). This final risk analysis document will inform development of risk mitigation components of translocation protocols, as well as a Biosecurity Risk Management Plan (BRMP) consistent with Australia's national guidelines for development of generic aquatic biosecurity plans (Sub-Committee on Aquatic Animal Health 2017). Thus the outcomes of the RA can be used for several purposes, such as design of biosecurity plans for

hatcheries and grow out farms, planning for diagnostic testing and surveillance programs, mitigation of risks posed by various pathogen introduction pathways such as treatment of incoming water, treatment of effluent water, quarantine and testing of broodstock, disposal of dead animals, disinfection of tanks, pipes and equipment, control of people/vehicle movements, disinfection of people/vehicles, routine disease surveillance, investigation and reporting of suspected disease outbreaks, assessment of disease risk posed by bringing new stock onto the site, and generic or specific plans for responses to disease incursions.

2.0 Commodity description

The species considered during this risk assessment included all life stages of spiny lobsters (Family Palinuridae) in general, and tropical rock lobsters (*Panulirus ornatus*) in particular. The commodities under consideration include broodstock *P. ornatus* originally domesticated in the University of Tasmania Institute for Marine and Antarctic Studies (IMAS), which have been returned to a biosecure hatchery owned by Ornatas Pty Ltd at Toomulla Beach, QLD. These have been supplemented by additional wild caught broodstock *P. ornatus* captured from the waters around Mackay, Cairns and Townsville. After quarantine, these adult lobsters will form the basis of a broodstock selective breeding programme which aims to domesticate a minimum of 4 broodstock populations of 10 lobsters each held in separate recirculation aquaculture systems (J Blair, personal communication, 15 September 2021).

Larval rearing and production of juvenile *P. ornatus* will be undertaken from these broodstock within the same hatchery at Toomulla Beach. The other translocation that will be considered in this RA is movement of settled *P. ornatus* juveniles (post-puerulus stage, 1-50 grams) from within the hatchery directly to grow out rafts in QLD, NT and WA. It will be assumed that the broodstock, larvae (pre settlement phyllosoma), puerulus and juvenile stages of *P. ornatus* will be maintained within the biosecure hatchery at Toomulla Beach at all times prior to the translocation.

3.0 Hazard Description

The next step in the RA process is to develop a comprehensive list of the relevant hazards (disease agents) to be analysed. This document will consider various hazards of lobsters that have been reported from Australia and overseas, including several that are under official control in Australia (Table 1). The national list of reportable diseases of aquatic animals in Australia is available online at https://www.agriculture.gov.au/animal/aquatic/reporting/reportable-diseases, while QLD, NT and WA have their own lists of notifiable diseases of fish and crustaceans which generally reflect the national list, but with some local differences. (Table 1). Most of the state disease lists are also available online, for example for QLD see https://www.legislation.qld.gov.au/view/html/inforce/current/act-2014-007#sch.1, and for WA see https://www.agric.wa.gov.au/bam/fish-diseases, while the NT list reflects the national list (M Barton, personal communication 28/10/2019). The disease status of each state is presented by DAWE (2020).

The criteria for consideration during the hazard identification process were as follows:

For the proposed commodity, the following questions were considered:

1. Whether lobsters (Families *Palinuridae*, *Nephropidae*) are known to be susceptible and/or could potentially be infected by the disease agent,
2. If the disease agent is "under official control", by its listing in State or National lists of reportable diseases (Table 1), or

3. If the disease agent could conceivably cause a detrimental impact to industry or the environment if infected tropical rock lobsters were translocated into new areas where the disease agent is absent.

For any disease agent, if the answers to any of these questions was 'yes', it was classified as a potential hazard (Figure 1) and was included in a list of diseases to be considered during initial hazard identification (Table 2).

Table 1. National list of reportable diseases of crustaceans (i.e. diseases under official control).

Australia's National List of Reportable Diseases of Aquatic Animals 2021	**Listed in the OIE *Aquatic Animal Health Code (2021)***	**Present in Australia**	**Present in QLD[1]**	**Present in NT[1]**	**Present in WA[1]**
CRUSTACEANS					
1. Infection with Taura syndrome virus (TSV)	✓				
2. Infection with White spot syndrome virus (WSSV)	✓	✓	✓		
3. Infection with yellow head virus genotype 1 (YHV1)	✓				
4. Gill-associated virus (GAV)		✓	✓	✓	✓
5. Infection with infectious hypodermal and haematopoietic necrosis virus (IHHNV)	✓	✓	✓	✓	
6. Infection with *Aphanomyces astaci* (crayfish plague)	✓				
7. Infection with *Macrobrachium rosenbergii* nodavirus (MrNV)	✓	✓	✓		
8. Infection with infectious myonecrosis virus (IMNV)	✓				
9. *Monodon* slow growth syndrome (MSGS)					
10. Infection with *Hepatobacter penaei* (necrotising hepatopancreatitis) (NHP)	✓				
11. Acute hepatopancreatic necrosis disease (AHPND)	✓				
12. Infection with *Enterocytozoon hepatopenaei* (EHP)					
13. Infection with Decapod iridescent virus 1 (DIV1)					

[1] The disease status of each state as presented in DAWE (2020).

Each disease agent identified as a potential hazard was then critically evaluated. Any disease agents considered likely to cause detrimental impacts in Australia based on one or more of the following criteria were classed as priority diseases of concern (hazards) that required detailed risk assessment. The additional criteria used included whether:

- the disease agent would be expected to cause a distinct pathological effect in an infected population; and/or
- it would be expected to cause economic harm (e.g. increased mortality, reduced growth rates, decreased product quality, loss of market access, increased costs); and/or
- it would be expected to cause damage to the environment and/or endemic species (defined as either native species that occur naturally in Australia waters, or species that were introduced into Australia and are now considered to be acclimatised).

If the disease agent did not meet these additional criteria, it was considered to represent a negligible risk and was excluded from the priority list of diseases of concern requiring detailed risk analysis and required no further assessment. The process used for decision making in relation to the hazard identification process is summarised below in Figure 1.

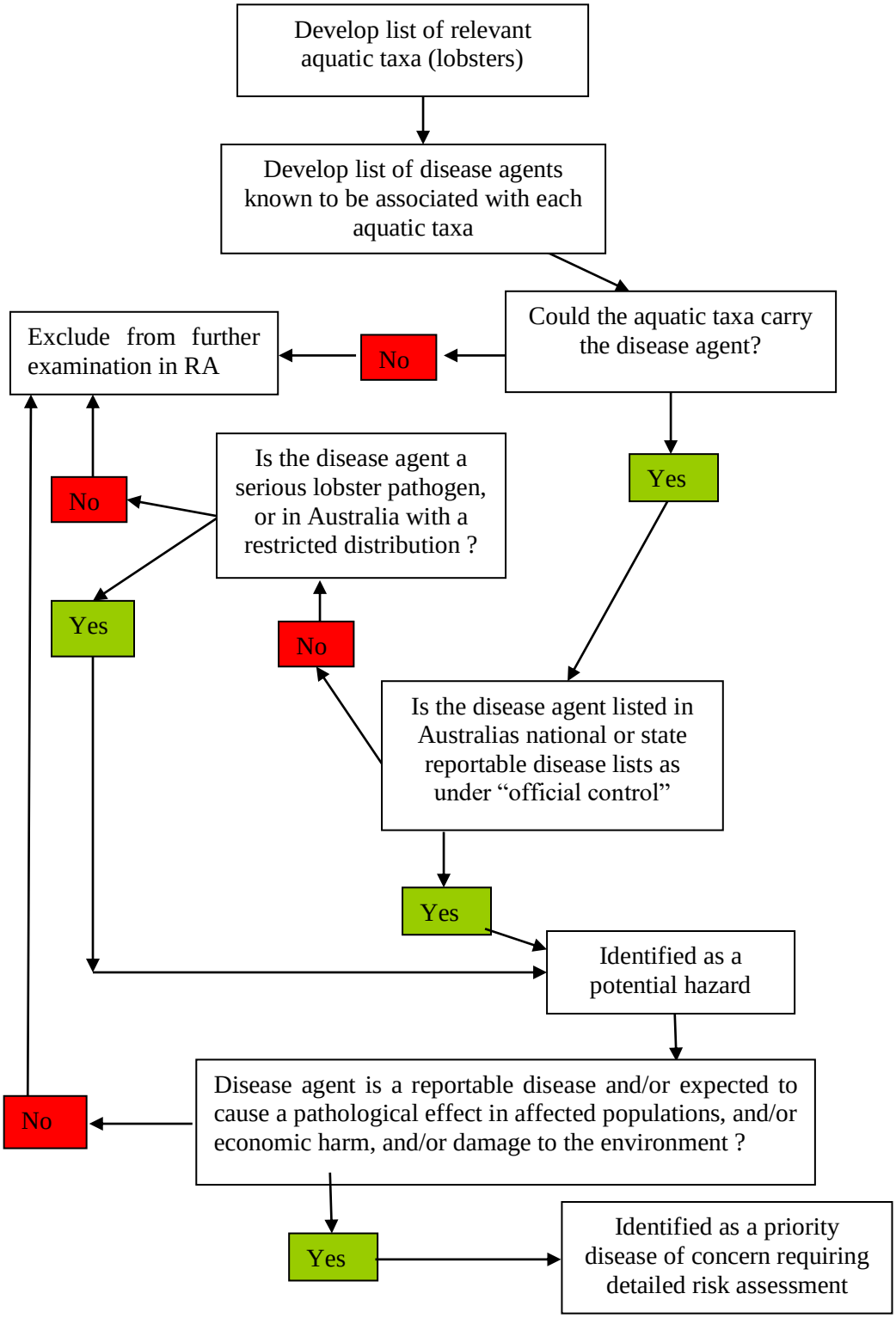


Figure 1. Flow chart showing the decision making process used to identify potential hazards in the hazard identification step.

Table 2. The list of diseases to be considered during initial hazard identification.

Disease (or disease agent)	Present in Australia	State where disease has been reported[A]	Under official control in Australia[1,2,3,4,5,6,7,8]	State where disease is listed	Main crustacean host groups
Viruses					
Baculoviral midgut gland necrosis virus (BMNV)	No	-	Yes	SA, NT	Prawns
Gill-associated virus (GAV)	Yes	QLD, NSW, NT, WA	Yes	Vic, Tas, SA, WA, NT, ACT	Prawns
Infection with Decapod iridescent virus 1 (DIV1)	No	-	Yes	All states	Prawns, crayfish, freshwater shrimp
Infection with *Homarus gammarus* nudivirus (HgNV)	No	-	No	-	Clawed lobsters (F. *Nephropidae*)
Infection with infectious hypodermal and haematopoietic necrosis virus (IHHNV)	Yes	QLD, NSW, NT	Yes	NSW, Vic, Tas, SA, WA, NT, ACT	Prawns
Infection with infectious myonecrosis virus (IMNV)	No	-	Yes	All states	Prawns
Infection with *Macrobrachium rosenbergii* nodavirus (white tail disease) (MrNV)	Yes	QLD	Yes	NSW, Vic, Tas, SA, WA, NT, ACT	Freshwater prawns
Infection with *Panulirus argus* virus 1 (PaV1)	No	-	No	-	Spiny lobsters (F. *Palinuridae*)
Infection with undescribed endemic viruses	Yes	-	No	-	Spiny lobsters
Infection with Taura syndrome virus (TSV)	No	-	Yes	All states	Prawns, crabs
Infection with white spot syndrome virus (WSSV)	Yes	QLD	Yes	All states	All decapods
Infection with yellow head virus genotype 1 (YHV1)	No	-	Yes	All states	Prawns
Monodon slow growth syndrome (MSGS)	No	-	Yes	All states	Prawns
Spawner isolated mortality virus (SMV)	Yes	QLD	Yes	SA, NT	Prawns, crayfish
Spherical baculovirus (*Penaeus monodon*-type virus)	Yes	QLD, NSW, NT	Yes	NT	Prawns
Tetrahedral baculovirus (*Baculovirus penaei*)	No	-	Yes	NT	Prawns

[1] https://www.agriculture.gov.au/animal/aquatic/reporting/reportable-diseases#crustaceans,
[2] https://www.legislation.qld.gov.au/view/html/inforce/current/act-2014-007#sch.1
[3] https://www.legislation.nsw.gov.au/#/view/act/2015/24/sch2
[4] https://dpipwe.tas.gov.au/biosecurity-tasmania/animal-biosecurity/animal-health/notifiable-animal-diseases
[5] https://www.pir.sa.gov.au/biosecurity/aquatics/aquatic_diseases
[6] https://www.agric.wa.gov.au/bam/fish-diseases
[7] https://agriculture.vic.gov.au/biosecurity/animal-diseases/notifiable-diseases
[8] https://www.legislation.act.gov.au/di/2018-33/

Disease (or disease agent)	Present in Australia	State where disease has been reported[A]	Under official control in Australia[1,2,3,4,5,6,7,8]	State where disease is listed	Main crustacean host groups
Bacteria					
Acute hepatopancreatic necrosis disease (AHPND)	No	-	Yes	QLD, NSW, Vic, SA, NT, ACT	Prawns
Ectocommensal filamentous bacteria (*Leucothrix* spp., *Thiothrix* spp.)	Yes	All states	No	-	Decapods
Gaffkemia (infection with *Aerococcus viridans*)	No	-	No	-	Clawed lobsters
Infection with *Hepatobacter penaei* (necrotising hepatopancreatitis) (NHP)	No	-	Yes	All states	Prawns
Milky haemolymph disease of spiny lobsters (*Panulirus* spp.) (MHD-SL)	No	-	Yes	QLD, WA	Spiny lobsters
Shell disease (Tail fan necrosis)	Yes	All states	No	-	Spiny lobsters
Vibriosis (incl. white leg disease (*Aquimarina* sp.), luminous vibriosis from *Vibrio harveyi*)	Yes	All states	No	-	Prawns, lobsters
Fungi					
Fusarium spp.	Yes	All states	No	-	Lobsters, prawns
Infection with *Enterocytozoon hepatopenaei* (EHP)	No	-	Yes	NSW, Vic, Tas, SA, NT, ACT	Prawns
Microsporidosis (*Ameson, Hepatospora, Myospora, Thelohania* spp.)	Yes	QLD, WA	Yes	WA, ACT	Decapods
Protozoa					
Atkinsiella spp., *Halioticida* spp., *Haliphthoros* spp., *Lagenidium* spp.	Yes	All states	No	-	Lobsters, prawns
Ectocommensal ciliates (*Carchesium* spp., *Epistylis* spp., *Vorticella* spp., *Zoothamnium* spp.)	Yes	All states	No	-	Decapods
Haplosporidosis	Yes	QLD	Yes	SA, WA, NT	Jelly prawns
Infection with *Hematodinium* spp.	Yes	QLD, Vic	No	-	Crabs, lobsters
Infection with *Aphanomyces astaci* (Crayfish plague)	No	-	Yes	All states	Freshwater crayfish
Neoparamoeba pemaquidensis	Yes	Tas	No	-	Clawed lobsters, finfish
Scuticociliate disease (*Anophryoides* spp., *Lynnia* spp., *Mesanophrys* spp., *Orchitophrya* spp.)	Yes	?	No	-	Decapods
Metazoa					
Ectoparasitic barnacles (*Octolasmis* spp.)	Yes	All states	No	-	Decapods
Nemerteans (*Carcinonemertes* spp.)	Yes	?	No	-	Decapods
Parasitic barnacles (*Lernaeodiscus* spp., *Parthenopea* spp., *Sacculina* spp.)	Yes	All states	No		Crabs, clawed lobsters, squat lobsters
Parasitic helminths (cestodes, digeneans, nematodes)	Yes	All states	No		Decapods
Non-infectious diseases					
Moult death syndrome, Pink lobster syndrome, Turgid lobster syndrome	Yes	All states	No	-	Lobsters, decapods

[A] The disease status of each state as presented in DAWE (2020). ? denotes uncertainty regarding identity and distribution of these disease agents within Australia.

3.1 Elimination of insignificant diseases.

Preliminary hazard identification for the diseases reported from lobsters, including the diseases of crustaceans under official control in Australia identified at least 39 diseases of potential concern (Table 2). These included 15 viral diseases, 7 bacterial diseases, 3 fungal diseases, 7 protozoan diseases and 4 groups of metazoan disease agents, as well as 3 diseases of non-infectious aetiology (Table 2).

However, as mentioned above the unrestricted risk posed by several of the disease agents or groups of disease agents listed in Table 2 is likely to be either negligible, or within the acceptable level of protection (ALOP), due to the fact that they do not meet the additional criteria used to define significant hazards. Section 3.1 contains a brief discussion of the reasons why these particular disease agents have been excluded from further assessment. However, it must also be considered that knowledge regarding the health status of aquatic animals in Australia is incomplete, particularly in the case of rock lobsters, and that various new diseases will continue to emerge as time goes on (Gaughan 2002). Furthermore, the threat from invasive pests and diseases continues to increase directly in line with increasing volumes of international trade (Diggles 2017b, 2020a, Scott-Orr et al. 2017). Because of this, it must be acknowledged that this hazard list represents a snapshot of the known disease situation at the time of publication. The hazard list and the RA will therefore need to be updated on a regular basis as new information becomes available.

3.1.1 Viruses

Disease agents excluded

Several of the viral diseases included in Table 2 are caused by significant internationally notifiable pathogens of crustaceans which are listed on Australia's national and state lists of notifiable diseases of aquatic animals. However, a number of these are not known to infect lobsters or be present in Australia at this time (DAWE 2020), and for this reason they can be excluded from the priority list of diseases of concern, and require no further assessment. The exotic viral diseases of crustaceans that do not infect lobsters and thus require no further assessment include: Baculoviral midgut gland necrosis (BMNV), infection with Decapod iridescent virus 1 (DIV1), infectious myonecrosis virus (IMNV), Taura syndrome virus (TSV), yellowhead virus genotype 1 (YHV1), *Monodon* slow growth syndrome (MSGS), and tetrahedral baculovirus (*Baculovirus penaei*). Similarly, there are several viruses listed in Table 2 which occur in Australia and cause notifiable diseases in penaeid prawns, but are not known to infect spiny lobsters. These include gill associated virus (GAV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), *Macrobrachium rosenbergii* nodavirus (MrNV), spawner isolated mortality virus (SMV) and spherical baculovirus (*Penaeus monodon* -type viruses). Because these disease agents have not been reported to infect spiny lobsters, they will not be considered further in this RA.

Another virus listed in Table 2 which infects lobsters overseas is *Homarus gammarus* nudivirus (HgNV). This was the first virus reported from naturally infected clawed lobsters (Family *Nephropidae*) when it was detected in hatchery reared European lobsters (*Homarus gammarus*) being grown out in a sea-based container culture system (Holt et al. 2019). The virus was observed inside distinctive intranuclear inclusions within affected hepatopancreocytes of juvenile lobsters in both the hatchery and at sea, however the virus was not associated with disease, and infected lobsters appeared apparently healthy (Holt et al. 2019). Prevalence of the inclusions in juvenile lobsters peaked at 17% around 39 weeks post-

deployment to sea, with prevalence dropping to 0% after 104 weeks post deployment. However, the prevalence in juveniles retained within the hatchery peaked at 53% at 104 weeks without any signs of clinical disease, suggesting that the nudivirus had low (or no) virulence and did not cause disease (Holt et al. 2019). Because of this, and the fact that HgNV is not known from Australia at this time, it can be excluded from the priority list of diseases of concern, and requires no further assessment.

Viral disease agents retained for detailed assessment

All decapod crustaceans, including clawed and spiny lobsters, are known to be susceptible to infection with white spot syndrome virus (WSSV) (see Chang et al. 1998a, Rajendran et al. 1999, Musthaq et al. 2006, Ross et al. 2019a). Because WSSV is a serious internationally notifiable pathogen of crustaceans which is known to occur in some parts of QLD (Diggles 2020a, 2020c), it will be retained for detailed risk assessment. *Panulirus argus* virus 1 (PaV1) was the first virus to be reported from naturally infected lobsters. This virus was found to cause systemic infection, disease and mortality of wild *P. argus* in the Florida Keys from 1999/2000 (Behringer et al. 2001, Shields and Behringer 2004, Li et al. 2008a), and was subsequently found causing disease in many areas throughout the Caribbean (Butler et al. 2008, Shields 2011, Behringer et al. 2011). Infection of *P. argus* by PaV1 is characterised by a milky colouration of the haemolymph and lethargy of the host, with the virus initially infecting fixed phagocytes in the hepatopancreas, prior to spreading to cells of the connective tissues (Shields and Behringer 2004, Li et al. 2008a, Shields 2011). Mortality rate due to PaV1 is higher in juvenile lobsters with a carapace length of less than 16 mm (Butler et al. 2008), and healthy lobsters have been observed to avoid infected conspecifics (Behringer et al. 2006, 2011). PaV1 is the most significant naturally occurring disease currently known from spiny lobsters. This virus causes significant disease in juvenile lobsters, and despite its absence from Australia its emergence in the Caribbean appears a useful case study that is relevant to the proposed translocations due to the paucity of information about lobster diseases in Australia. Because of this, PaV1 will be retained for detailed risk assessment, as will an example of an undescribed endemic virus.

3.1.2 Bacteria

Disease agents excluded

Several of the bacterial diseases included in Table 2 are significant pathogens of crustaceans which are listed on Australia's national and state lists of notifiable diseases of aquatic animals. However, two of these are only known to infect prawns and are not known to be present in Australia at this time (DAWE 2020), and for these reasons they can be excluded from the priority list of diseases of concern, and require no further assessment. The exotic bacterial diseases that require no further assessment include acute hepatopancreatic necrosis disease (AHPND), and necrotizing hepatopancreatitis caused by infection with *Hepatobacter penaei* (see Lightner and Redman 1994).

All crustaceans have a "normal" bacterial flora (or microbiome) which is moved whenever the host is translocated. There are also facultative bacterial pathogens such as *Aerococcus* spp. and *Vibrio* spp. that are considered to be ubiquitous in aquatic environments (Austin and Austin 2007), but certain strains of which can cause disease and mortalities in aquatic animals that are stressed, injured and/or exposed to adverse environmental conditions. For example, *Aerococcus viridans* var. *homari*, the causative agent of gaffkemia disease in clawed lobsters held in onshore holding facilities in North America (Stewart 1975,

Brock and Lightner 1990, Lavallée et al. 2001), is also pathogenic to experimentally infected *Panulirus interruptus* (see Schapiro et al. 1974) and may also occur naturally in *P. argus* (see Bobes et al. 1988). The later stages of gaffkemia infection cause 'red tail' in clawed lobsters, after the bacterium gains entry into the host lobster through damaged areas on the host exoskeleton, however it cannot infect lobsters through intact cuticle or via ingestion (Shields 2011). *Aerococcus viridans* var. *homari* is therefore considered an opportunistic bacterium which invades compromised hosts only when they are damaged or stressed due to unfavorable conditions. Because of this, and the fact it is not reported from Australia, nor is gaffkemia a notifiable disease, *A. viridans* var. *homari* will not be considered further in this RA.

Similarly, shell disease is caused by various chitinoclastic bacteria which infect damaged areas of the carapace, particularly in adult lobsters (Brock and Lightner 1990, Diggles et al. 2002a, Evans 2003). Various permutations of shell disease lesions exist and they are usually most obvious on the ventral part of the tail fan and other areas of the carapace in contact with bottom surfaces, or subject to injury (Evans 2003, Stephens et al. 2003). Such an example includes tail fan necrosis disease of southern rock lobster (*Jasus edwardsii*) (see Geddes et al. 2003, Musgrove et al. 2005, Zha et al. 2018), and epizootic shell disease in *Homarus americanus* (see Quinn et al. 2012, Ranson et al. 2018, Reardon et al. 2018). Shell disease lesions including tail fan necrosis are caused by invasion of cuticular injuries by the normal bacterial flora (Porter et al. 2001) and thus can usually be eliminated by simple husbandry measures such as improved tank cleanliness and reduced handling, as shown by evidence that tail fan necrosis is more prevalent in areas where *J. edwardsii* are handled repeatedly in lobster pots (Freeman and MacDiarmid 2009). Similarly, improved tank cleanliness can control epibiont fouling with filamentous bacteria such as *Thiothrix* spp. and *Leucothrix mucor* which were problematic in cultured larvae and juveniles of the American lobster (Fisher et al. 1976, 1978), as well as cultured phyllosoma larvae and juveniles of spiny lobsters (Kittaka 1997, Handlinger et al. 1999, 2000, Diggles 1999, 2000, Diggles et al. 2002a, Diggles and Handlinger 2003, Bourne et al. 2004, 2006, Payne et al. 2008). For this reason, and the fact that the agents responsible are ubiquitous in the marine environment, shell disease (including tail fan necrosis) and filamentous bacteria will not be considered further in this RA.

The phyllosoma larvae of spiny lobsters have a long larval rearing period spanning several months (range 4-7 months for the tropical *P. ornatus*, 12-22 months for the temperate *Jasus edwardsii*, see Hall et al. 2013). During this time, they are particularly prone to microbial diseases due to infection by various species of *Vibrio* spp. (see Hall et al. 2013) and *Aquimarina* sp. (see Ooi et al. 2020). For example, Diggles et al. (2000) reported mortalities of cultured phyllosoma larvae of *Sagmariasus* (=*Jasus*) *verreauxi* in New Zealand caused by infection with a luminescent strain of *Vibrio harveyi*. Diseased phyllosoma larvae were opaque, had small red spots throughout the body and glowed in the dark (Diggles et al. 2000). The hepatopancreas was atrophied and the hepatopancreatic tubules had extensive bacterial plaques, resulting in mortalities of up to 75% after 4 weeks, with mortality rates highest in injured larvae (Diggles et al. 2000). Bourne et al. (2004) also found that mortalities in phyllosoma larvae of *P. ornatus* cultured in Australia were largely due to microbial diseases including infection by *V. harveyi*, *V. parahaemolyticus*, and various other groups of marine bacteria which occur naturally in seawater and biofilms. Research has found a very dynamic microbial community in phyllosoma larviculture systems (Payne et al. 2006), with the detection of *Vibrionaceae* at the end of some larval trials coinciding with mass phyllosoma mortality due to infection with *Vibrio harveyi*, which demonstrated that bacterial proliferation in biofilms can act as a reservoir for potentially pathogenic bacteria (Bourne et al. 2006, Webster et al. 2006). In contrast, *Vibrio* spp. were rarely detected in the microbiome of *Panulirus* spp.

phyllosomas sampled from the wild (Payne et al. 2008), indicating that these bacteria tend to proliferate in the intensive aquaculture environment.

Other marine bacteria besides *Vibrio* spp. can also be associated with disease in cultured phyllosoma larvae. For example, a recent study by Ooi et al. (2020) found that the gram negative marine bacterium *Aquimarina* sp. was associated with white leg disease in damaged pereiopods of cultured phyllosomas of *P. ornatus*, eastern rock lobster (*Sagmariasus verreauxi*), and slipper lobster (*Thenus australiensis*). Various species of *Aquimarina* (a member of the *Flavobacteriaceae*) occur in seawater, sediment and other marine environments in many tropical and subtropical environments (Zheng et al. 2016). Some species, such as *A. hainanensis*, show strong chitinolytic activity and can be associated with disease outbreaks in a wide range of larval crustaceans including not only spiny lobsters (Ooi et al. 2020), but prawns (Zheng et al. 2016), brine shrimp (*Artemia franciscana*), freshwater shrimp (*Caridina multidentata*), marine crabs (*Portunus trituberculatus, Scylla serrata*) (see Midorikawa et al. 2020) and the clawed lobster *Homarus americanus* (see Quinn et al. 2012, Ranson et al. 2018, Reardon et al. 2018). The *Aquimarina* sp. isolated from *P. ornatus, S. verreauxi* and *T. australiensis* shared 98.1–100% sequence similarity with *A. hainanensis* (see Ooi et al. 2020).

Over the last two decades of development of the spiny rock lobster aquaculture industry, successful phyllosoma larviculture has been achieved only following attempts to control and manipulate the microbiome of the larval rearing environment and live feeds, with improved survival of phyllosomas occurring following disinfection of water and *Artemia* nauplii using direct application of ozone, sometimes in combination with probiotics, to ensure that beneficial (rather than potentially pathogenic) bacteria dominate the bacterial community in rearing tanks (Ritar et al. 2006, Høj et al. 2009, Jensen et al. 2011, Hall et al. 2013, Powell and Scolding 2018). Similar attempts to study and manipulate the microbiome have also been undertaken for juvenile spiny and clawed lobsters (Ooi et al. 2017, Holt et al. 2020). These data from various studies together are consistent with vibriosis and *Aquimarina* sp. disease of phyllosoma larvae and juvenile lobsters being associated with opportunistic bacteria which are normal components of the marine microflora, but which can invade sensitive early life stages of spiny lobsters under intensive culture conditions due to cuticular damage, suboptimal water quality and/or system cleanliness resulting in dysbiosis of the microbiome (Ooi et al. 2017, Holt et al. 2020). Therefore, because of the likely ubiquitous distribution of *Aquimarina* spp. and *Vibrio* spp., the facultative nature of this disease process, and the fact they are not listed diseases and amenable to control, they will not be considered further in this RA.

Bacterial disease agents retained for detailed assessment

A bacterial disease called milky haemolymph disease (MHD) of spiny lobsters was first reported in spiny lobsters (*Panulirus versicolor, P. ornatus, P. homarus*) collected from the Bay of Lang Co in central Vietnam and held in a nearby onshore holding system (Diggles 2008, Lightner et al. 2008, Callinan and Corsin 2009, Nunan et al. 2010). The disease was caused by massive systemic infections with a rickettsia-like organism (RLO) and briefly threatened the developing spiny lobster farming industry in Vietnam (Lightner et al. 2008, Callinan and Corsin 2009, Nunan et al. 2010). Because of this, milky haemolymph disease in spiny lobsters (MHD-SL) was temporarily listed in the OIE Aquatic Animal Health Code (OIE 2009) as 'under study' for possible listing as a notifiable disease of *Panulirus* spp., and an OIE disease card was developed to help clarify the case definition (see OIE 2008, 2009, Nunan et al.

2010). The disease was problematic in net-pen-reared spiny lobsters in Vietnam which were being fed a variety of fresh foods including trash fish, molluscs and decapod crustaceans acquired locally from fishers (OIE 2008), being responsible for around 20% of mortalities observed during the grow out cycle (Callinan and Corsin 2009). Despite its absence from Australia, the emergence of MHD-SL in Asia appears a useful case study that is relevant to the proposed translocations (particularly the proposed grow out of juvenile *P. ornatus* in sea rafts in Cone Bay in WA), due to the paucity of information about lobster diseases in Australia. Because this RLO causes significant disease in juvenile lobsters, and as this disease remains listed in both QLD and WA, MHD-SL will be retained for detailed risk assessment.

3.1.3 Fungi

Disease agents excluded

The deuteromycete fungus *Fusarium solani* is an opportunistic pathogen which causes disease in a wide range of crustaceans, usually after they are exposed to stressors such as damage or water pollution (Brock and Lightner 1990, Edgerton et al. 2002). *Fusarium solani* has been reported from both clawed lobsters and spiny lobsters (Evans 2003, Stephens et al. 2003, Shields 2011), including *Panulirus homarus* with black gill disease in Indonesia (Sudewi et al. 2018a), and *Panulirus cygnus* from WA where it caused melanised lesions on the abdomen, uropods, telson and pereopods (McAleer and Baxter, 1983). In both these cases, as is usual with nearly all fungal diseases, environmental factors (related to poor water quality or poor husbandry) facilitated the disease outbreaks in confined crustaceans, and it has been found that these opportunistic infections can be prevented by enhanced cleanliness (Bower et al. 1994, Diggles 2001, Evans 2003, Stephens et al. 2003, Hall et al. 2013). Because these fungal agents are ubiquitous opportunistic pathogens which probably already occur throughout NA, and they are not likely to threaten the health of wild or confined crustaceans under normal environmental conditions when husbandry and water quality are optimal, they will not be considered further. However, the potential impact of these fungi on fecundity should be noted during development of broodstock husbandry protocols.

Fungal disease agents retained for detailed assessment

Microsporidians are obligate, intracellular eukaryotic parasites that infect every major group of animals including crustaceans (Lom and Dykova 1992, Stentiford et al. 2016). Molecular phylogenetic analysis of microsporidia has demonstrated that they are related to the Fungi, either as a basal branch of the Fungi or as a sister group (Keeling 2014). Microsporidians are known pathogens of crustaceans, particularly penaeid prawns, but also crabs, freshwater crayfish and spiny lobsters (Herbert 1988, Hudson et al. 2001, Kiryu et al. 2009, Ding et al. 2016, Stentiford et al. 2016, Small et al. 2019b). One microsporidian listed in Table 2 (*Enterocytozoon hepatopenaei*, or EHP) is a significant pathogen of prawns which is listed internationally as well as on Australia's national and state lists of reportable diseases of aquatic animals. However, *E. hepatopenaei* is known to infect only penaeid prawns and is not known to be present in Australia at this time (DAWE 2020), and for this reason it can be excluded from the priority list of diseases of concern, and requires no further assessment. However, a microsporidian was reported infecting the tail muscle of *Panulirus cygnus* and *P. ornatus* from the Torres Strait and WA, but infections were extremely rare (<0.1%, see Dennis and Munday 1994). Because these disease agents are known to occur in spiny lobsters from both QLD and WA, but not other areas, and microsporidosis is a notifiable disease in WA and the ACT, other microsporidians (excluding EHP) will be retained for detailed assessment.

3.1.4 Protozoa

Disease agents excluded

Wild caught lobsters can harbour a wide range of protozoan parasites, ectocommensals and symbionts including various ectocommensal fouling organisms such as sessile epibiont ciliates (e.g. *Carchesium* spp., *Epistylis* spp, *Vorticella* spp., *Zoothamnium* spp.) which attach together with filamentous bacteria to the gills, carapace and egg masses (Brock and Lightner 1990, Payne et al. 2008, Shields 2011). These organisms have direct lifecycles and thus can be readily translocated into aquatic animal populations in new geographic areas, particularly as these symbionts have low host specificity. Sessile ciliates are ubiquitous on the eggs, gills and carapaces of all marine crustaceans, including spiny lobsters (Shields 2011), These ectocommensal symbionts were problematic in the earlier years of spiny lobster larval rearing, probably due to the fact that tank culture tends to trap excessive nutrients compared to the oligotrophic environment normally inhabited by larval lobsters (Payne et al. 2008). In recent years development of techniques which control the microbial flora of larval rearing systems through ozone disinfection of water and treatment of *Artemia* nauplii have greatly reduced their importance (Ritar et al. 2006, Høj et al. 2009, Jensen et al. 2011). Furthermore, these opportunistic epibiont ciliates have ubiquitous distributions, and this, together with the fact that they are not listed diseases, means that sessile epibiont ciliates will not be considered further in this RA.

Aphanomyces astaci is a member of a group of fungus-like organisms called water moulds (Class Oomycetes). Although long regarded as fungi, this group are now considered protists and are included among the Protista in a group called the Stramenopiles or Chromista. *Aphanomyces invadans* causes crayfish plague, which is an internationally notifiable fungal disease (particularly of freshwater crayfish) included in Table 2 and which is therefore also listed on Australia's national and state lists of notifiable diseases of aquatic animals. However, *A. astaci* is an exotic disease that is known not to be present in Australia at this time (DAWE 2020), and only occurs in freshwater. For these reasons, *A. astaci* can be excluded from the priority list of diseases of concern, and requires no further assessment.

Some of the other water moulds listed in Table 2 include the genera *Atkinsiella* spp., *Halioticida* spp., *Haliphthoros* spp. and *Lagenidium* spp. . All are ubiquitous opportunistic saprophytes often found in soil and plants and which are also known to occur on or in crustacea (Brock and Lightner 1990), including spiny lobsters where they typically infect the eggs or larvae, but seldom adult lobsters (Shields 2011). For example, *Atkinsiella panulirata* is an oomycete described from phyllosoma of *Panulirus japonicus* (see Kitancharoen and Hatai 1995). Another oomycete *Haliphthoros mildfordensis* was associated with mortalities of confined postlarval clawed lobsters (Fisher et al. 1975, 1976, 1978, Nilson et al. 1975) and spiny lobsters (Diggles 2001). Infection by *Haliphthoros* sp. caused mortalities in puerulus and juvenile *Jasus edwardsii* held in tanks in New Zealand (Diggles, 2001). The fungus only infected the gills of lobsters less than 30 mm carapace length, with infected lobsters exhibiting morbidity, including lethargy, loss of appetite and death (Diggles 2001). *Halioticida noduliformans* was found in co-infections with *Lagenidium* spp. in eggs and gills of European lobsters (*Homarus gammarus*) being farmed in the UK, with the fungi causing pathology of the egg mass, likely leading to reduced fecundity (Holt et al. 2018). It appears that all these water moulds have ubiquitous distributions, and this, together with the fact that they are not listed diseases, means that *Atkinsiella* spp., *Halioticida* spp., *Haliphthoros* spp. and *Lagenidium* spp. will not be considered further in this RA. However, the potential impact of water moulds on fecundity should be noted during development of broodstock husbandry protocols.

Systemic infections with amoebae (*Neoparamoeba pemaquidensis*) were reported as one of several potential disease causing processes affecting dying American lobsters (*H. americanus*) in Long Island Sound, New York in 1999 (Mullen et al. 2004). However, a combination of several environmental stressors including pollution and hypoxia are thought to have contributed to immune suppression that increased susceptibility to amoebae and other non-specific disease processes (Dove et al. 2004, Shields 2013). Subsequent investigations have found that increasingly prevalent conditions such as amoebiasis, systemic calcinosis, and epizootic shell disease in *H. americanus* along the north east coast of the USA are likely to be symptomatic of multifactorial environmental processes affected by pollution and climate change (Castro et al. 2012, Shields 2013, 2019, Reardon et al. 2018). Because infection by *N. pemaquidensis* appears to be part of a multifactorial immune suppression syndrome of American lobsters that is associated with environmental change (increasing temperature), and anthropogenic decline (exposure to contaminants) resulting in a dysbiotic bacterial community (Shields 2013, 2019), this disease does not appear to be relevant to the proposed translocations, and hence will not be considered further.

Protozoan disease agents retained for detailed assessment

The remaining protozoan disease agents listed in Table 2 are all known to be significant pathogens of crustaceans, are present in Australia, and some cause listed notifiable diseases. For example, haplosporidians are histozoic and coelozoic parasites of a variety of freshwater and marine invertebrates, particularly of molluscs, but some haplosporidians also infect crustaceans (Reece et al. 2004, Nunan et al. 2007, Azevedo and Hine 2016). Besides the molluscan disease agents *Bonamia* spp. and *Minchinia* spp. which cause notifiable diseases in Australian oysters, haplosporidosis (due to infection by other haplosporidians besides *Bonamia* and *Minchinia*) is also a reportable disease in WA and the NT (Table 2). While infection of crustaceans by haplosporidians is rare (Azevedo and Hine 2016), disease due to infection by haplosporidians has been reported overseas in blue crabs (*Callinectes sapidus*), spot prawns (*Pandalus* spp.), whiteleg shrimp (*Penaeus vannamei*) and European green crabs (*Carcinus maenas*) by Newman et al. (1976), Meyers et al. (1994), Nunan et al. (2007) and Stentiford et al. (2013), respectively. Within Australia, it has recently become known that haplosporidians infect jelly prawns (*Acetes sibogae australis*) in Moreton Bay in QLD (Diggles 2020a, Diggles et al. submitted). Because of the limited knowledge of the disease status of palinurid lobsters in Australia at this time, the known presence of haplosporidians in QLD waters, and the fact that haplosporidosis is listed as a notifiable disease in SA, WA and the NT, haplosporidians that infect crustaceans will be retained for detailed assessment.

Dinoflagellates in the genus *Hematodinium* are parasites of wild marine decapod crustaceans, particularly crabs and lobsters (Shields 1994, Stentiford and Shields 2005, Small 2012), however, they are also known to be problematic in other cultured crustaceans such as mud crabs *Scylla serrata* (see Li et al. 2008b) and palaemonid prawns (Xu et al. 2010). *Hematodinium australis* is known to infect a number of species of economically important crabs in QLD, including *Portunus pelagicus*, *Scylla serrata* and *Trapezia areolata* (see Shields 1992, Hudson and Lester 1994, Hudson and Shields 1994, Hudson and Adlard 1994, 1996). *Hematodinium* sp. has also been reported from sand crab (*Ovalipes australiensis*), spider crab (*Leptomithrax gaimardii*) and red bait crabs (*Plagusia chabrus*) from Port Phillip Bay, Victoria (Gornik et al. 2013). Because these disease agents are known to occur in Australia (particularly in QLD), and they are widely recognised as significant pathogens of both wild and cultured crustaceans (Stentiford and Shields 2005, Shields et al. 2006, Shields 2012, Small 2012), *Hematodinium* spp. will be retained for detailed assessment.

Various species of crustaceans, including lobsters are known to be susceptible to systemic infection by scuticociliates which can infect the haemolymph and other tissues (Morado and Small 1995, Metz and Hechinger 2021). For example, Small et al. (2005a) reported the presence of a histophagous ciliate infection in the clawed Norway lobster (*Nephrops norvegicus*) in Scotland. The ciliate was morphologically similar to scuticociliates in the genus *Mesanophrys*, but was genetically related more closely to *Orchitophyra stellarum*, and caused hemocytopenia, degeneration and necrosis of the myocardial heart muscle, and extensive infiltration of many organs particularly the gills (Small et al. 2005a). Another scuticociliate, *Anophryoides haemophilia*, infects captive American lobsters (*Homarus americanus*) causing "bumper car" disease (Cawthron et al. 1996, Greenwood et al. 2005). This disease agent was found at low prevalences of less than 1% in wild *H. americanus*, but was responsible for occasional mass mortalities and significant (10-15%) pre-processing mortality in lobsters held in onshore processing facilities (Lavallée et al. 2001). Scuticociliate infections usually result in significant disease in captive adult lobsters, but they can also cause disease in wild crustaceans (Morado et al. 1999, Metz and Hechinger 2021) and therefore could represent a threat not only to captive *P. ornatus* broodstock, but also other crustaceans in areas where juvenile *P. ornatus* are reared in grow out rafts. For these reasons, scuticociliates will be subjected to detailed risk assessment.

3.1.5 Metazoa

Disease agents excluded

Wild crustaceans, including lobsters, harbour a range of metazoan parasites, commensals and symbionts including amphipods, barnacles, and larval stages of various parasitic helminths with multi-host lifecycles (digenea, cestodes, nematodes) that infect fishes as the second intermediate host or final host (Shields et al. 2006, Shields 2011). Gooseneck barnacles, *Octolasmis* spp., use a range of decapod crustaceans as hosts, including spiny lobsters where they attach to the exoskeleton and gills (Shields et al. 2006, Shields 2011, Sudewi et al. 2018a). *Octolasmis angulata* has been recorded from *Panulirus versicolor* from WA (Jones 2004), while balanomorph barnacles such as *Paralepas* have also been reported (Jones 2003). Several helminths are known to use spiny lobsters as intermediate hosts including microphallid trematodes, larval tetraphyllidean cestodes in spiny lobsters from the Great Barrier Reef, and nematodes in *Jasus edwardsii* (see Shields 2011). Furthermore, egg predatory nemerteans *Carcinonemertes* spp. (Campbell et al. 1989, Shields and Kuris 1990) and amphipods, cf. *Parapleustes* spp. infest the egg clutches of at least 3 species of spiny lobsters, whilst other assorted parasitic copepods and miscellaneous metazoan symbionts can occur on the egg clutches or gills (Shields 2011). With few notable exceptions, all of these parasites occur naturally in healthy wild lobsters, and do not appear to be associated with any significant harm to their hosts (Shields et al. 2006, Diggles 2011, Shields 2011). As these do not appear to cause any significant harm to their hosts under normal environmental conditions, and because none of them cause any listed diseases, these various parasite species require no further assessment.

However, there is one group of metazoan parasites which are known to cause significant morbidity of their wild crustacean hosts, most notably parasitic rhizocephalan barnacles (e.g. *Heterosaccus* spp., *Loxothylacus* spp., *Sacculina* spp.), which are best known for parasitically castrating crabs (Boschma 1955, Weng 1987, Shields and Wood 1993, Murphy and Goggin 2000, Walker 2001, Gurney et al. 2006, Glenner et al. 2008). Rhizocephalan barnacles have recently been reported from clawed lobsters (Boyko and Williams 2020) and squat lobsters (Williams et al. 2019), hence given the limited knowledge of the disease status of wild lobsters in Australia (including a recent discovery of rhizocephalans infecting squat

lobsters *Munidopsis* spp. and *Galacantha* spp. in the Ningaloo Canyons, see https://schmidtocean.org/cruise-log-post/barnacles-like-youve-never-seen-before/), it is possible that broodstock *P. ornatus* from some areas of Australia could be infected by rhizocephalan barnacles. These parasites can cause significant reductions in fecundity (Shields and Wood 1993) or even complete castration which would make broodstock useless for breeding purposes. So while rhizocephalan barnacles will not be considered further in this RA because they are not listed disease agents and are not associated with mass mortalities, given their potential impact on fecundity, these parasites should be noted during development of broodstock husbandry protocols. Thus, for the various reasons described above, none of the metazoan diseases listed in Table 2 will be retained for detailed assessment.

3.1.6 Non-infectious diseases

While spiny lobsters are known to be affected by some non-infectious diseases, including moult death syndrome (Bowser and Rosemark 1981), turgid lobster syndrome (Diggles et al. 2002a, Evans 2003), and pink lobster syndrome (Shields et al. 2006, Shields 2011), these syndromes are related to husbandry conditions in captivity and are not known to be caused by infectious disease agents. Various other poorly characterised syndromes are also known in captive spiny lobsters in Asia, including big head syndrome, and separate head syndrome, the latter having some clinical characteristics of turgid lobster syndrome (Jones 2015). However, because non-infectious diseases do not threaten the health of wild crustaceans under normal environmental conditions, they will not be considered further.

3.2 The priority diseases of concern to be considered in the RA

After elimination of the insignificant diseases from Table 2, the remaining 8 diseases listed in Table 3 are considered to be diseases of potential concern that are relevant to the proposed translocations, and which therefore require detailed risk assessment.

Table 3. The list of priority diseases of concern to be considered in the detailed risk assessment.

Disease agent	Disease agent is infectious	Agent or strains confined to certain regions	Agent is under official control in Australia	Agent occurs in North QLD	Expected to cause significant disease
CRUSTACEANS					
Viruses					
Infection with *Panulirus argus* virus 1 (PaV1)	✓	✓	No	No	✓
Infection with undescribed endemic viruses	✓	✓	No	?	✓
Infection with white spot syndrome virus (WSSV)	✓	✓	✓	No	✓
Bacteria					
Milky haemolymph disease of spiny lobsters (*Panulirus* spp.) (MHD-SL)	✓	✓	✓	No	✓
Fungi					
Microsporidosis	✓	?	✓	✓	✓
Protozoa					
Haplosporidosis	✓	?	✓	?	✓
Infection with *Hematodinium* spp.	✓	?	No	✓	✓
Scuticociliate disease	✓	?	No	?	✓

4.0 The methodology used for the risk assessments

By virtue of the inherent uncertainty in relation to the incomplete knowledge of spiny lobster diseases both internationally and particularly within Australia, reporting of the findings of this RA will be based on a qualitative assessment of the risks involved. The qualitative RA method addresses risk in a standardised manner (Diggles and Arthur 2010, DAFF 2011, Diggles 2011, 2017b, 2020b, OIE 2021a) utilising a series of internationally recognised risk assessment processes which evaluate the risk of introduction (release), establishment (exposure) and spread (consequences) of each hazard through various specific pathways (Diggles and Arthur 2010, DAFF 2011, OIE 2021a). Individual risk assessments are conducted for each hazard of concern, with the nomenclature and risk estimation matrixes used in this risk assessment being adapted from those used in previous risk analyses (e.g. Diggles 2011, 2017b, 2020b).

Briefly, the likelihood of release and exposure of a hazard is combined with the consequences of establishment and spread to arrive at an overall risk estimation for each hazard of concern. If the risk estimation is considered to exceed an Appropriate Level of Protection (ALOP) of "very low" (annual probability between 1 in 20 and 1 in 100 years), risk mitigation methods will be required to reduce the risk to acceptable levels below the ALOP (probability of occurrence less frequent than 1 in 100 years). Bearing in mind some of the intended uses of this RA are to inform translocation protocols, biosecurity plans and standard operating procedures (SOPs), the RA will employ an adaptation of the risk rating system used in the national Aquaculture Farm Biosecurity Plan generic guidelines document (Sub-Committee on Aquatic Animal Health 2017). More detail on each step in the process is included below.

4.1 Release assessment

After defining the hazards of concern (see Table 3), the next step in the RA is to identify the potential release pathways/mechanisms of entry of hazards into the hatchery and the waters of QLD, WA and NT. Five specific release pathways are being considered for the detailed risk analysis. These include:

1. Broodstock: Released into the hatchery in north QLD.
2. Water: Taken into the hatchery in north QLD
3. Juveniles: Released into the waters of QLD
4. Juveniles: Released into the waters of the NT
5. Juveniles: Released into the waters of WA

The likelihood estimations (Table 4) that a hazard would be successfully translocated and released via a particular pathway will then be determined through qualitative assessments based on information available in the scientific (and other) literature, unpublished data, as well as the professional judgment of the analyst. Indicative annual probability ranges for each category are from DAFF (2003). These are for guidance only and probability will vary depending on the extent of disease surveillance in each jurisdiction, the frequency of translocations, and quantities of animals translocated. The risk rating system used is as per the national Aquaculture Farm Biosecurity Plan generic guidelines document (Sub-Committee on Aquatic Animal Health 2017). The risk assessment will be concluded if the release assessment determines that the likelihood of release of a particular hazard is negligible (OIE 2021a). Likelihood estimates are made based on "worst case" (unrestricted) situations which DO NOT take into

account biosecurity protocols that are often used in hatcheries (e.g. UV irradiation of intake water, disinfection of equipment).

Table 4. Nomenclature for the qualitative likelihood estimations used in the RA.

Risk Rating	Likelihood	Definition	Annual Probability	Event Horizon
5	High (H)	The event would be very likely to occur	$0.7 > P \leq 1$	Every 1-2 years
4	Moderate (M)	The event would occur with an even probability	$0.3 > P \leq 0.7$	Every 2-4 years
3	Low (L)	The event would be unlikely to occur	$0.05 > P \leq 0.3$	Every 4-20 years
2	Very Low (VL)	The event would be very unlikely to occur	$0.01 > P \leq 0.05$	Every 20-100 years
1	Extremely low (EL)	The event would be extremely unlikely to occur	$0.001 > P \leq 0.01$	Every 100-1000 years
0	Negligible (N)	The event would almost certainly not occur	$0 > P \leq 0.001$	>1000 years

4.2 Exposure assessment

The exposure assessment examines the likelihood of the environment and aquatic animals in the receiving jurisdictions being exposed to the hazards via the release pathways, and determines the likelihood of the establishment and spread of the hazards. The likelihood of exposure depends on several factors relating to the capacity of the hazard to survive in the environment, the availability of susceptible hosts, the ease of infection of susceptible hosts, and the likelihood of subsequent transmission of infection to others within a population. In determining the likelihood of establishment and spread, the following key factors were considered relevant:

- Availability and density of susceptible hosts
- Management of potential hosts and biosecurity protocols for local host populations
- Contiguity of host populations and presence of competent vectors
- Climatic and environmental suitability of zone
- Likelihood of early detection/eradication
- Methods of establishment and spread and rate of transmission in a population

Some additional considerations included:

1. *Route of Infection:* Viable disease agents must be ingested by a susceptible host or otherwise come into contact with susceptible host species. Infection may occur via the digestive tract, through direct contact with contaminated water via the external surfaces and gills, and/or
2. *Infective Dose:* The pathogen load carried by the commodity is also important as there must be sufficient quantities of viable infective stages to induce an infection following ingestion or contact with the disease agent via the external surfaces or gills.

Once a hazard is released into the environment, the likelihood of whether it would survive, infect susceptible hosts, and become established will be expressed qualitatively using the likelihood estimations in Table 4, based on information available in the scientific (and other) literature, unpublished data, as well as the professional judgment of the analyst. The likelihoods for the release and exposure assessments will then be combined using the matrix of 'rules' for combining descriptive likelihoods, to arrive at a likelihood of establishment and spread, as shown in Table 5.

Table 5. Matrix of rules for combining descriptive likelihoods for the release and exposure assessments to arrive at a likelihood of establishment and spread.

		Likelihood of release					
		High	**Moderate**	**Low**	**Very Low**	**Extremely low**	**Negligible**
Likelihood of exposure	**High**	High	Moderate	Low	Very Low	Extremely low	Negligible
	Moderate	Moderate	Low	Low	Very Low	Extremely low	Negligible
	Low	Low	Low	Very Low	Very Low	Extremely low	Negligible
	Very Low	Very Low	Very Low	Very Low	Extremely low	Extremely low	Negligible
	Extremely low	Extremely low	Extremely low	Extremely low	Extremely low	Negligible	Negligible
	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible

The risk assessment for a particular hazard will be concluded if the exposure assessment determines that the probability of establishment is likely to be negligible (OIE 2021a).

4.3 Consequence assessment

The consequence assessment estimates the likely magnitude of the consequences of establishment and/or spread of a hazard into a new region, including the possible effects of disease agents on aquatic animals, the environment, industry and the economy. The qualitative terms that were used to describe the consequences of establishment of an unwanted disease agent in this RA are defined in Table 6, These descriptions are based on information available in other RAs (Jones and Stephens 2006, Biosecurity Australia 2009, Diggles and Arthur 2010, Diggles 2011, 2017b, 2020b), the scientific literature, unpublished data, as well as the professional judgment of the analyst. The risk rating system used is from the national Aquaculture Farm Biosecurity Plan generic guidelines document (Sub-Committee on Aquatic Animal Health 2017).

For each hazard of concern, the consequence assessment determined the likelihood of occurrence and the associated impact for each of two main outbreak scenarios. Either:

1. The disease agent becomes established and spreads throughout the receiving jurisdiction. This scenario assumes that if an agent were to establish it would eventually spread to its natural geographical limits, or;

2. An index case occurs (an animal becomes infected), but the agent does not persist in the environment.

Only the first scenario will be considered to represent establishment of the disease agent, because the second scenario would most likely go undetected.

Table 6. Definition of terms used to describe consequences of establishment of disease agents.

Risk Rating	Consequence	Definition
5	Extreme	Establishment of disease would cause substantial biological and economic harm at a regional or even national level, and/or cause serious and irreversible harm to the environment.
4	High	Establishment of disease would have serious biological consequences (high mortality or morbidity) and would not be amenable to control or eradication. Such organisms would significantly harm economic performance at a regional level and/or cause serious environmental harm which is most likely irreversible.
3	Moderate	Establishment of disease would cause significant biological consequences and may not be amenable to control or eradication. Such organisms could harm economic performance at a regional level on an ongoing basis and/or may cause significant environmental effects, which may or may not be irreversible.
2	Low	Establishment of disease would have moderate biological consequences and would normally be amenable to control or eradication. Such organisms may harm economic performance at a local level for some period and/or may cause some environmental effects, which would not be serious or irreversible.
1	Very Low	Establishment of disease would have mild biological consequences and would be amenable to control or eradication. Such organisms may harm economic performance at a local level for a short period and/or may cause some minor environmental effects, which would not be serious or irreversible.
0	Negligible	Establishment of disease would have no significant biological consequences and would require no management. The disease would not affect economic performance at any level and would not cause any detectable environmental effects.

The risk assessment for a particular hazard was concluded if the consequence assessment determined that the consequences of introduction are negligible (OIE 2021a).

4.4 Risk estimation

Risk estimation is the final step involved with each assessment and was used to determine whether the extent of the unrestricted risk presented by each hazard to the aquatic animals, environment, industries and community of the receiving jurisdiction is sufficient to require risk management. 'Unrestricted risk' means the estimated risk if the various hazards were to be translocated with NO risk management measures in place. Risk was assessed using the risk estimation matrix in Table 7 which uses a combination of the qualitative answers given for the combined likelihoods of release and exposure and the significance of the consequences of establishment of a disease agent to provide an unmitigated risk estimate, ranging from 'negligible' through to 'extreme'. The appropriate level of protection (ALOP) that was adopted in this RA was expressed in qualitative terms. as "very low" (annual probability between 1 in 20 and 1 in 100 years). This definition of ALOP, and its illustration by way of a risk estimation matrix, is shown below in Table 7, together with the relevant risk scores (Sub-Committee on Aquatic Animal Health 2017).

Table 7. Risk estimation matrix showing the ALOP utilized for this RA (White squares = very low risk).

Combined likelihood of release and exposure	Negligible	Very Low	Low	Moderate	High	Extreme
High	Acceptable risk 5	Very low risk 5	Moderate risk 10	High risk 15	High risk 20	Extreme risk 25
Moderate	Acceptable risk 4	Very low risk 4	Low risk 8	Moderate risk 12	High risk 16	High risk 20
Low	Acceptable risk 3	Acceptable risk 3	Very low risk 6	Low risk 9	Moderate risk 12	High risk 15
Very low	Acceptable risk 2	Acceptable risk 2	Acceptable risk 4	Very low risk 6	Low risk 8	Moderate risk 10
Ext. Low	Acceptable risk 1	Acceptable risk 1	Acceptable risk 2	Acceptable risk 3	Very low risk 4	Very low risk 5
Negligible	Acceptable risk 0	Acceptable risk 1	Acceptable risk 2	Acceptable risk 3	Acceptable risk 4	Very low risk 5

Consequences of establishment and spread

If either the likelihood of establishment and spread, or the significance of the consequences of establishment and spread of disease agent were considered negligible, extremely low or very low, the unrestricted risk would be within the ALOP (i.e. acceptable risk, risk score ≤ 6), and there would be no need to implement any additional risk management steps. However, if the unrestricted risk estimation for any disease agent was determined to be unacceptable (i.e. exceeded a risk score of 6), additional risk mitigation measures will be required to reduce the risk estimate back to within the ALOP. The risk estimation matrix and tables are colour coded for clarity, using the following: Extreme risk (risk score 21-25) = purple, High risk (risk score 15-20) = red, Moderate risk (risk score 10-14) = orange, Low risk

(7-9) = yellow, at the ALOP = white (risk scores 4-6, acceptable risk). Below the ALOP = acceptable risk (green) (Table 7).

4.5 Risk mitigation

For any hazards with unrestricted risk estimation rankings that exceed the ALOP of "very low", (i.e., anything above a risk score of 6 representing an annual probability between 1 in 20 and 1 in 100 years), additional risk mitigation measures will need to be identified to reduce the risk estimate back to an acceptable level (i.e. to reduce the risk to a probability of occurrence less frequent than 1 in 100 years). The risk mitigation processes examined as part of this RA process relate only to option evaluation together with an appraisal of the utility of each option for reducing risks to within the ALOP. The risk mitigation options identified can then be used as a basis to inform development of biosecurity protocols and standard operating procedures for collection of broodstock, operation of the hatchery facilities in QLD, as well as testing and certification procedures and translocation protocols for broodstock and juveniles in order to reduce any such risks to acceptable levels. The options thus identified could then form the basis of a consultation process that engages Government and stakeholders to evaluate the biosecurity risks involved with unrestricted pathways/mechanisms/risk factors for entry with a view towards developing translocation protocols incorporating appropriate risk mitigation steps that would reduce the risks identified to an acceptable level for each jurisdiction.

5.0 Risk Assessment

5.1 Infection with *Panulirus argus* virus 1 (PaV1) (including undescribed endemic virus)

5.1.1 Aetiologic agent: *Panulirus argus* virus 1 (PaV1), an unclassified, unenveloped, intranuclear double stranded DNA virus with an icosahedral nucleocapsid approximately 182 ± 9 nm in dimension (Behringer et al. 2001, Shields and Behringer 2004, Behringer et al. 2011). Recent phylogenomic analysis supports the classification of PaV1 with several other nucleocytoplasmic large DNA viruses within a distinct Family *Mininucleoviridae* (see Subramaniam et al. 2020).

5.1.2 Under official control in Australia: No **Zoonotic:** No

5.1.3 Australias status: *Panulirus argus* virus 1 (PaV1) has never been recorded in Australia and is considered exotic.

5.1.4 Epizootiology

In 1999, while sampling juvenile Caribbean spiny lobster (*Panulirus argus*) populations in the Florida Keys, lethargic, moribund juvenile (15-55 mm carapace length (CL)) lobsters with chalky white haemolymph that did not clot were reported for the first time (Behringer et al. 2001). Haemolymph smears were negative for parasites and gram-negative bacteria, but histopathology of lobster tissues showed numerous abnormal haemocytes and spongy connective tissue with hypertrophied nuclei with marginated chromatin (Shields and Behringer 2004, Li et al. 2008a). The nuclei of affected cells contained eosinophilic Cowdry Type-A viral inclusions with virtually all of the host hyalinocytes and semigranulocytes destroyed in heavily infected individuals (Behringer et al. 2001), however circulating granulocytes remained largely unaffected (Shields and Behringer 2004). TEM confirmed the disease was due to infection of the affected cells by an unenveloped, intranuclear icosahedral virus with a nucleocapsid approximately 182 ± 9 nm in dimension (Behringer et al. 2001) which was named *Panulirus argus* virus 1 (PaV1) by Shields and Behringer (2004). The affinities of the new virus were unclear, as although similar in some respects to iridoviruses and herpesviruses, viral morphogenesis was completed entirely within the host cell nucleus rather than in the cytoplasm (Shields and Behringer 2004, Subramaniam et al. 2020). Virions were found to assemble in the nucleus and in clinically infected lobsters large aggregations of virions were also found free in the haemolymph, with large numbers of infected haemocytes present in fixed phagocytes and throughout the haemolymph spaces of the heart, hepatopancreas, gills, hindgut, abdominal muscle and other tissues of mesodermal origin (Shields and Behringer 2004, Li et al. 2008a, Behringer et al. 2011).

PaV1 infected juvenile *P. argus* occurred at 75% - 100% of the nursery habitat sites (n = 14 sites) surveyed in the middle and lower Florida Keys, with the prevalence of overt infections (based on gross signs of disease in lethargic animals with milky haemolymph) ranging from 6 to 8% but with certain sites reaching prevalences of 37-40% (Behringer et al. 2001, Shields and Behringer 2004) and even up to 60% in certain "hot spots" (Behringer et al. 2011). Pathology of infected spiny lobsters showed a marked depletion of reserve inclusions in cells of the hepatopancreas and spongy connective tissues as lethargic, clinically diseased lobsters ceased feeding and eventually died between 30-90 days post-infection (Shields and Behringer 2004, Butler et al. 2008, Behringer et al. 2011). It appears that natural PaV1 infections impair moulting and alter the physiology and immunocompetency of *P. argus*, with PaV1

infected lobsters having nearly 50% higher prevalence of gill infestation by ectocommensal ciliates (*Epistylis* spp. and *Zoothamnium* spp.) compared to non-infected lobsters (Pascual-Jiménez et al. 2012). As the course of disease progresses, digestive enzyme levels are altered in the hepatopancreas indicating a loss of digestive efficiency (Herrera-Salvatierra et al. 2019), dysbiosis of gut microbiota occurs (Zamora-Briseno et al. 2020), and glycogen levels decrease suggesting mortality is ultimately due to nutritional deficiency and metabolic failure (Behringer et al. 2011, Herrera-Salvatierra et al. 2019). Clinical disease thus seems progressive and 100% fatal, and as such it has been estimated that at least 24% of settled *P. argus* puerulus never recruit to the Florida rock lobster fishery minimum size, due to mortality from PaV1 infection (Moss et al. 2013, Subramaniam et al. 2020).

Subsequent studies found the PaV1 occurred in *P. argus* populations throughout many areas of the Caribbean, including not only the Florida Keys, but also Mexico, Belize, Bahamas, Cuba, Dominican Republic, Honduras, Panama, Puerto Rico, and the US Virgin Islands (Butler et al. 2008, Huchin-Mian et al. 2008, 2009, Behringer et al. 2011, Moss et al. 2013, Davies et al. 2020a). Prevalences of PaV1 in adult *P. argus* in the wild were highest in Puerto Rico (17%), Florida Keys (11%) and Cuba (6.3%), whilst the virus was not detected in adult lobsters from several south eastern Caribbean locations (Martinique, St. Kitts, Venezuela, and Curacao), nor from Bermuda (Moss et al. 2013). However, sampling only adult lobsters is likely to underestimate PaV1 prevalence (Moss et al. 2013), because in the wild clinical disease and mortality is observed only in juvenile lobsters 15-55 mm CL (Shields and Behringer 2004, Lozano-Alvarez et al. 2008, Davies et al. 2020a). Indeed, the development of molecular diagnostic tools (Montgomery-Fullerton et al. 2007, Clark et al. 2018) found that while the planktonic phyllosoma larvae do not appear to be infected (Lozano-Alvarez et al. 2015) the transparent early settlement *P. argus* post-larvae (puerulus) could be infected with PaV1 (Moss et al. 2012, Lozano-Alvarez et al. 2015). Infected juveniles that do not experience clinical disease and survive into adulthood remain subclinically infected (Behringer et al. 2012b, Moss et al. 2013).

The highly social behaviour of juvenile *P. argus* was originally thought likely to facilitate transmission of disease (Behringer et al. 2001). However, in practice it was found that infected juveniles were more often found alone in dens and crevices (92% of individuals) compared to healthy juveniles (56-68% of individuals), and that this phenomenon was driven by changes in the behaviour of healthy lobsters, which apparently use olfactory cues to avoid cohabitation with diseased conspecifics (Behringer et al. 2001, 2006, Lozano-Alvarez et al. 2008, Candia-Zulbara et al. 2015). It appears that this social distancing generates "behavioural immunity" that can effectively suppress the onset of severe epizootics in wild *P. argus* populations (Butler et al. 2015, Butler and Behringer 2021), nevertheless PaV1 infections still have significant ecological as well as fisheries related ramifications beyond the elevated pre-recruit mortality (Behringer et al. 2008). Indeed, it has been demonstrated that certain fishery practices such as using lobster traps "baited" with live, sublegal-sized lobsters to socially attract other lobsters increases the risk of spreading PaV1 infections, and PaV1 infected lobsters have predictably been shown to be less effective as social attractants compared to uninfected lobsters (Behringer et al. 2012b).

PaV1 is horizontally transmissible and juvenile *P. argus* can be infected via inoculation, ingestion, direct contact between infected and unjnfected lobsters and via viral particles shed into seawater over short distances of around 2 meters at water temperatures between 19 and 30°C (Shields and Behringer 2004, Butler et al. 2008). Contact with infected lobsters and water-borne viral transmission are apparently the primary modes of transmission in nature (Behringer et al. 2011). Given that PaV1 has not been detected

in wild caught phyllosoma larvae, there is no evidence to date of vertical transmission of the virus and this is considered unlikely (Moss et al. 2012, Kough et al. 2015, Lozano-Alvarez et al. 2015, Clark 2017, Don Behringer, personal communication, 20 September 2021). Larger juvenile lobsters (32-55 mm CL) inoculated with infected haemolymph from diseased conspecifics can develop acute infections and up to 38% died within 80 days (Butler et al. 2008). In contrast, lobsters infected via natural routes such as contact or water-borne transmission generally take a longer time to die (up to 200 days), with mortality rate highly correlated with initial infective dose (higher initial dose = faster disease process) and host size (smaller lobsters are more susceptible to disease) (Butler et al. 2008, Behringer et al. 2011). Nevertheless, 52% of the smallest *P. argus* (6-16 mm CL) in contact and waterborne trials contracted the disease and 33% died within 4 months (Butler et al. 2008), while Clark (2017) found that 100% of juvenile *P. argus* (exposed to experimental PaV1 infection via the water (doses ranging between 1.5 x 10^8 and 2.6 x 10^9 viral copies per lobster) became subclinically infected. Three species of decapod crustaceans that naturally co-occur with wild *P. argus* (including spotted lobster *Panulirus guttatus*, stone crab *Menippe mercenaria*, and channel crab *Mithrax spinosissimus*) were refractory to infection after inoculation with PaV1 infected haemolymph, suggesting that PaV1 may be highly host specific to *P. argus* (see Butler et al. 2008), however it appears that a comprehensive assessment of the host range of PaV1 has not been undertaken, and the apparent persistence of PaV1 in oceanic waters suggests there may be as yet unidentified reservoir hosts in that environment, possibly in flotsam or floating *Sargassum* mats (Kough et al. 2015, Lozano-Alvarez et al. 2015).

5.1.5 Release assessment

PaV1 has never been recorded in Australia and is considered an exotic disease. However, the disease status of TRL populations in northern Australia is poorly known, and because of this there is a high likelihood that new (currently undescribed) endemic viruses could emerge at some stage in the future during development of the TRL industry in northern Australia in a scenario similar to the emergence of PaV1 in the Caribbean, and in Vietnam where unidentified intranuclear viral inclusions were visualised in diseased wild caught TRL puerulus (Diggles 2008). Inspections of supermarkets in various locations throughout QLD, the NT and WA over the past several years have also found evidence of retail sale of frozen TRL tails imported from Central and South American countries, particularly Brazil (BK Diggles, personal observations – photos available upon request). Given that PaV1 is endemic and widely distributed in *P. argus* populations throughout the Caribbean and Central America (Moss et al. 2013), and PaV1 has been detected at high prevalence (50%) in frozen imported *P. argus* tails originating from certain countries within this region (Huchin-Mian et al. 2009), the risk of PaV1 being present in frozen uncooked *P. argus* tails imported into Australia from Central and South America is non-negligible. It is known that many crustacean viruses remain viable and infectious in frozen products, and that these products can potentially spread these viruses to other countries through exports (Nunan et al. 1998, Durand et al. 2000, Hasson et al. 2006, Scott-Orr et al. 2017). Non-enveloped viruses, such as PaV1, are often more robust and persist for longer periods outside the host compared to enveloped viruses, as the lipid envelope is more easily damaged (Kough et al. 2015, Lin et al. 2020). Therefore, it is not surprising that Clark (2017) found that PaV1 remained viable and infectious for *P. argus* after freezing then thawing from -20°C, and she also found that free-virions of PaV1 remained viable and infective in seawater for at least 21 days.

The fact that PaV1 remains viable after freeze/thawing is significant, as it may remain viable in frozen uncooked *P. argus* exported from the Caribbean and South America that are now available for retail purchase at supermarkets in many parts of Australia. While the majority of imported lobster tails sold for human consumption are probably used for their intended purpose, it is well known that a significant proportion of recreational fishers in Australia use supermarket purchased seafood as bait or burley (Kewagama Research 2007, Future Fisheries Veterinary Service 2017, Senate 2017, Kantar Public 2019). Indeed, this is the most likely pathway by which other crustacean viruses such as WSSV have entered and established within the Australian environment (Scott-Orr et al. 2017, Oakey and Smith 2018, Oakey et al. 2019, Kantar Public 2019, Diggles 2020a, 2020c). Huchin-Mian et al. (2009) found that 50% of frozen uncooked *P. argus* tails exported from Belize into Mexico tested positive for PaV1 by cPCR, however in the absence of a testing program for lobster tails imported into Australia, it is unknown what proportion of frozen uncooked *P. argus* tails available for retail sale in supermarkets in Australia are positive for PaV1. In any case, the water temperatures in NA are suitable for survival of the pathogen (19-30°C) and this suggests that the risk of viable PaV1 being introduced into coastal waters around northern Australia via this particular pathway are non-negligible.

In contrast to WSSV, the range of crustaceans which have been reported to be susceptible to PaV1 infection is limited to *P. argus*, and the information available to date appears to suggest that PaV1 has high host specificity because attempts to infect three other decapod species were unsuccessful (Butler et al. 2008). However, very little experimental work has been done examining the full host range of PaV1, and it is possible that the virus persists in the environment in other, as yet unidentified reservoir species (Moss et al. 2012, 2013, Lozano-Alvarez et al. 2015). There appears to be little information published on inactivation of PaV1, however Clark (2017) noted that laboratory UV sterilizers have been demonstrated effective at inactivating PaV1 virions. Taking into account the information above, the risk of release is not negligible, and unrestricted likelihood estimations for the release of PaV1 (with estimates for a similar undescribed endemic virus in parentheses) into northern Australian waters via the various release pathways are provided below.

Release assessment for *Panulirus argus* virus 1 (PaV1)
(or a similar undescribed endemic virus, in parentheses)

Pathways	Via broodstock into hatchery in north QLD	Via water into hatchery in north QLD	Via juveniles into waters of QLD	Via juveniles into waters of the NT	Via juveniles into waters of WA
Likelihood of release	Very low (Moderate)	Extremely low (Moderate)	Very low (Moderate)	Very low (Moderate)	Very low (Moderate)

5.1.6 Exposure assessment

PaV1 can be horizontally transmitted by cohabitation with conspecifics at water temperatures between 19 and 30°C and the route of infection by this method is either through direct contact or via viral particles in the water surrounding infected lobsters (Shields and Behringer 2004, Butler et al. 2008). While the minimum infective dose via this pathway is unknown, it must be less than the 1.5 x 10^8 viral copies per lobster which was found to be 100% successful for infecting juvenile (25–35 mm carapace length) *P. argus* via the water by Clark (2017). Transmission via the water by co-habitation with infected lobsters was successful only over short distances of around 2 meters, such that it appears co-habitation is the

primary mode of transmission in nature (Behringer et al. 2011). Juvenile (19-34 mm carapace length) *P. argus* can also become infected via ingestion of tissues from diseased conspecifics, with 42% of all lobsters fed 1 gram of PaV1 infected tail muscle tissue showing pathological signs of disease, increasing to 83% of the smallest lobsters which suffered 33% mortality within 80 days (Butler et al. 2008).

The minimum infective dose for successful PaV1 infection should theoretically vary depending on lobster size, because larger lobsters are more refractory to infection (Shields and Behringer 2004), however the minimum infective dose to cause infection remains to be determined for both the *per-os* and waterborne routes. Furthermore, Clark (2017) was unable to determine the minimum infective PaV1 dose via the inoculation route, as 100% of *P. argus* became infected as shown by qPCR testing after being exposed to doses as low as a 10^{-6} serial dilution of haemolymph from a diseased *P. argus* (containing 7.37×10^4 viral copies). In that study 0.2 ml of undiluted haemolymph of a clinically diseased juvenile *P. argus* was found to contain 1.67×10^{11} viral copies (Clark 2017). This suggests that the viral load is such that 0.1 ml of haemolymph from a clinically diseased juvenile *P. argus* contains enough virus to theoretically infect around 1 million other juvenile lobsters via the injection route. Adult broodstock, however, would not be expected to be clinically diseased (Behringer et al. 2011), and thus would be expected to harbour lower viral loads.

Lobsters that survive infection with PaV1 appear to become life-long subclinical carriers of the virus into adulthood (Behringer et al. 2012b, Moss et al. 2013), however there is little evidence that vertical transmission occurs between broodstock, eggs and phyllosoma larvae (Moss et al. 2012, Lozano-Alvarez et al. 2015). Nevertheless, early settlement *P. argus* post-larvae (puerulus) are known to be susceptible to infection with PaV1, presumably via the waterborne route (Moss et al. 2012, Lozano-Alvarez et al. 2015), and PaV1 appears to be able to survive in seawater for periods of at least 21 days (Clark 2017). This means that without appropriate biosecurity, waterborne transmission of PaV1 from broodstock to puerulus and juvenile lobsters would appear likely within a confined hatchery situation. It is assumed that PaV1 has high host specificity, but this assumption is based on unsuccessful attempts to infect only 3 other species of decapods (two crabs and the congeneric spotted lobster *Panulirus guttatus*, see Butler et al. 2008). Hence, it remains to be determined if TRL native to Australia are susceptible to infection with PaV1. If they are, and infected juvenile TRL were translocated into new regions, native populations of TRL in northern Australia would be highly vulnerable as they would never have been naturally exposed to it and therefore would be completely naïve to PaV1 infection. Given that PaV1 could persist in lobster broodstock and theoretically be transmitted within hatchery environments, whilst environmental conditions are suitable for disease transmission in the wild in northern Australia, the risk of exposure and establishment is non-negligible, and the overall likelihood of exposure and establishment is considered to be **Very Low** for PaV1, and **Moderate** for a similar undescribed endemic virus.

5.1.7 Consequence assessment

Introduction and establishment of PaV1 (or a similar undescribed endemic virus, see Diggles 2008) into the coastal environment of northern Australia would likely have highly significant ramifications, both ecologically and financially, due to the fact that these diseases can cause significant mortality in wild lobsters. Furthermore, if other species of lobsters were found to be susceptible to PaV1, it remains to be seen whether they would actively avoid infected conspecifics in the wild as *P. argus* does, and in doing so reduce the risk of epizootics through "behavioural immunity" via social distancing (Behringer et al. 2006, Butler et al. 2015, Butler and Behringer 2021). If they did not socially distance, mortality rates of wild

lobsters in NA could greatly exceed the estimated 24% pre-recruit mortality rate reported for the Florida rock lobster fishery (Moss et al. 2013). Furthermore, even though PaV1 or similar undescribed viruses are not under official control, if any new viral disease was found causing mortality of wild lobsters in northern Australia, there is a high chance that its detection would necessitate intervention by government authorities and disruption to normal lobster fishery and aquaculture trade activities, if attempts were made to contain the infection and prevent its further spread into uninfected areas. However, once these disease agents are detected in the wild or in an inshore seacage aquaculture situation, depending on its host range and presence of reservoir hosts, there would appear to be little chance of eradication, meaning that the detrimental effects of their introduction and establishment may be permanent and irreversible. On the other hand, given that PaV1 (or a similar undescribed endemic virus) is not currently listed as an internationally notifiable disease, its emergence would be unlikely to have significant ramifications for international trade. Taking all of these factors into consideration, the establishment of these diseases could have serious biological consequences, would cause significant economic and environmental harm and would not be amenable to control or eradication, hence the consequences of introduction and establishment into the environment of northern Australia via the identified risk pathways would likely be **High** for PaV1, and **Moderate** for a similar undescribed endemic virus.

5.1.8 Risk estimation

The unrestricted risk associated with PaV1 (or a similar undescribed endemic virus) is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The unrestricted risk estimate for PaV1 does not exceed the ALOP for any of the pathways examined, suggesting that additional risk management is not required for this disease agent at this time. However, a testing program is recommended for lobster tails imported into Australia to determine what proportion of frozen uncooked *P. argus* tails available for retail sale in supermarkets in Australia are positive for PaV1, and research is encouraged in order to determine if TRL native to Australia are susceptible to infection with PaV1. Furthermore, the unrestricted risk estimate for a similar undescribed endemic virus exceeds the ALOP for all of the pathways examined, suggesting that additional risk management is required for these disease agents.

Risk estimate for *Panulirus argus* virus 1 (PaV1)
(or a similar undescribed endemic virus, in parentheses)

Pathways	Via broodstock into hatchery in north QLD	Via water into hatchery in north QLD	Via juveniles into waters of QLD	Via juveniles into waters of the NT	Via juveniles into waters of WA
Combined likelihood of release and exposure	Extremely low (Moderate)	Extremely low (Moderate)	Extremely low (Moderate)	Extremely low (Moderate)	Extremely low (Moderate)
Consequences of establishment	High (Moderate)	High (Moderate)	High (Moderate)	High (Moderate)	High (Moderate)
Risk estimation PaV1	Very low risk 4	Very low risk 4	Very low risk 4	Very low risk 4	Very low risk 4
Risk estimation undes. endemic virus	(Moderate risk 12)	(Moderate risk 12)	(Moderate risk 12)	(Moderate risk 12)	(Moderate risk 12)

5.2 Infection with white spot syndrome virus (WSSV)

5.2.1 Aetiologic agent: White Spot Syndrome Virus (WSSV), a double-stranded DNA virus of the genus Whispovirus within the family *Nimaviridea* (Mayo 2005, OIE 2021b). Numerous synonyms exist, including rod shaped nuclear virus of *Penaeus japonicus* (RV-PJ), penaeid rod-shaped DNA virus (PRDV), white spot baculovirus (WSBV), PmNOBIII, systemic ectodermal and mesodermal baculovirus (SEMBV) or PmNOBII and Chinese baculovirus (CBV) (Lightner 2003).

5.2.2 Under official control in Australia: All states **Zoonotic:** No

5.2.3 Australias status: Established in SE QLD and under official control. Australia was considered free of white spot disease (WSD) caused by infection with WSSV until late November 2016, when an outbreak of WSD was recorded in black tiger prawns (*Penaeus monodon*) cultured along the Logan River, in South East Queensland (DAF QLD 2017, Diggles 2020a). Subsequent surveillance found WSSV to also be present in wild crustaceans in northern Moreton Bay during the summer months (Oakey and Smith 2018, Oakey et al. 2019), and it appears that the virus has now established in a range of crustacean hosts in this region (Diggles 2020c).

5.2.4 Epizootiology

White spot disease first emerged in 1992-93 in several locations in Asia including Taiwan and Japan, though epidemiological evidence suggests the virus originated from China. Devastating epizootics in *Penaeus japonicus* in Japan in 1993 were due to a new rod shaped virus that was introduced with imported post larvae (PL) from China (Momoyama et al. 1994, Inouye et al. 1994, Nakano et al. 1994, Takahashi et al. 1994). At around the same time mortalities in farmed *P. japonicus*, *P. monodon* and *P. pencillatus* due to a similar disease were also occurring in Taiwan, perhaps from as early as 1992 (Chou et al. 1995). In Taiwan the disease was described as white spot syndrome by Chou et al. (1995) due to the presence of prominent white spots on the carapace of diseased prawns. The Taiwanese disease outbreaks were caused by a new rod shaped virus similar to that described in Japan (Chou et al. 1995) which was subsequently named white spot syndrome virus (WSSV) (Lightner 1996a).

Epizootic disease caused by rod-shaped viruses in cultured prawns displaying similar clinical signs to those reported in Japan and Taiwan were subsequently reported throughout Asia from China (1995), Thailand (1995), Korea (1998), India (1998), and the Philippines (1996) (Lightner 2003, Stentiford et al. 2012). Despite the absence of evidence of live prawn introductions from Asia to the Americas, WSSV was also diagnosed at several sites in 1995-1997 in captive wild prawns and freshwater crayfish and in cultured prawns in the eastern and southeastern United States (Nunan et al. 1998, Durand et al. 2000). Soon after, the WSSV panzootic reached prawn farms in southeastern Europe (1997), the Middle East (1999), India (Rajendran et al. 1999), and even central and South America in early 1999 (Lightner 2003). By mid to late 1999, WSSV was causing major losses in Ecuador, and by 2000-01, export of prawns from Ecuador was down nearly 70% from pre-WSSV levels (Lightner 2003).

Viable WSSV has been recovered from crustacean tissues (including commodity prawns) frozen at -20 or -70°C after months to several years storage and used to successfully infect susceptible crustaceans (Wang et al. 1998, Soto et al. 2001, McColl et al. 2004, Hasson et al. 2006, Bateman et al. 2012, RM Overstreet, personal communication, Nov 2009). Free WSSV also remains viable after storage at -18 to -20°C for up

to several years, but survival and viability upon thawing is reduced from that stored at -70°C (RM Overstreet, personal communication, Nov 2009). It is thus known that a significant percentage of WSSV remains viable after at least one freeze-thaw cycle during commercial freezing, storage and transport of uncooked prawn commodities (McColl et al. 2004, Biosecurity Australia 2009). This apparently resulted in the introduction of WSSV into the Americas following importation of frozen prawn products from WSSV-affected areas of Asia and the value-added reprocessing of frozen prawns for the US market in coastal processing plants (Lightner et al. 1997, Nunan et al. 1998, Durand et al. 2000, Lightner 2003, Hasson et al. 2006). WSSV also reached Spain and Australia in 1999-2001 in frozen prawns (Lightner 2003, Biosecurity Australia 2009). In both cases successful containment and eradication was reported, and for both events the importation and use of WSSV infected frozen uncooked prawns as a fresh feed for broodstock crustaceans was implicated as the route of introduction (Lightner 2003, Biosecurity Australia 2009). Frozen commodity prawns sampled from supermarkets were also confirmed as a route of entry for WSSV into Europe (Bateman et al. 2012). In Australia, an incursion of WSSV was detected in broodstock *P. monodon* and mud crabs (*Scylla serrata*) fed frozen imported prawns at an aquaculture hatchery in Darwin Harbour in December 2000. In that case wild mud crabs and prawns adjacent to the hatchery outlet were also transiently infected with WSSV, but over time this infection apparently was self limiting and subsequent testing showed the virus did not become established in Darwin Harbour (East et al. 2004, 2005). This incident stimulated interest in tightening quarantine controls for frozen commodity prawns imported into Australia, based mainly on increased processing as well as testing for WSSV and yellowhead virus (Biosecurity Australia 2009). However, by 2015 importers were evading the enhanced quarantine testing programs on a massive scale, leading to dumping of large quantities of WSSV-infected prawns into Australian supermarkets (Future Fisheries Veterinary Service 2017, Senate 2017, Scott-Orr et al. 2017, Diggles 2020a).

Studies have found that up to 27% of recreational fishers in south east QLD use frozen imported prawns purchased from supermarkets as bait or burley (Kantar Public 2019). With such a direct pathway for introducing large quantities of WSSV infected product into the coastal environment, it was not entirely unexpected when an outbreak of WSD was eventually recorded in black tiger prawns (*Penaeus monodon*) cultured along the Logan River, in South East Queensland in late November 2016 (DAF QLD 2017, Diggles 2020a). Subsequent surveillance found WSSV to also be present in wild crustaceans in northern Moreton Bay during the summer months at locations remote from the affected prawn farms (Oakey and Smith 2018, Oakey et al. 2019). Despite concerted efforts to eradicate WSSV from the prawn farms on the Logan River, surveys of wild prawns, crabs and other potential WSSV vectors conducted in the autumn months of 2020 found that WSSV had established in a range of crustacean hosts in the Moreton Bay White Spot Biosecurity Area in south east QLD (Diggles 2020c). The establishment of WSSV in this zone has severely impacted prawn fisheries in Moreton Bay, which were Australia's largest suppliers of commercially gathered bait prawns. All prawn products from Moreton Bay are now subject to quarantine measures consistent with Australia's domestic ALOP for prawn products originating from regions where WSSV occurs, namely a requirement for sanitary measures equivalent to cooking or exposure to high levels of gamma irradiation (50 kilogray [kGy]) (Diggles 2020a). Disruption of the bait prawn supply continues to affect recreational fisheries Australia-wide due to reduced availability and increased cost of domestic bait prawns, which has led to a perverse economic incentive for recreational fishers to use more imported prawns from supermarkets as bait (Kantar Public 2019, Diggles 2020a, 2020c).

WSD of farmed penaeids is characterised by high and rapid mortality, sometimes accompanied by gross signs in moribund prawns of white, initially circular, spots in the cuticle (calcium deposits), and overall red body coloration (Lightner 1996a). The white spots are not pathognomonic, however, as they can also be caused by environmental insults, bacterial infection (Wang et al. 2000) and moult -related calcification (Diggles et al. 2020). Diseased cultured penaeids cease feeding, and moribund prawns are observed swimming near the surface at the edge of rearing ponds. Rapid and severe mortality up to 100% often follows within 3 or 4 days after the first appearance of gross signs (Chou et al. 1995). Pathological changes associated with WSD include characteristic basophilic nuclei and necrosis of subcuticular epithelium and other tissues of ectodermal and mesodermal origin (Lo et al. 1997). Infection of crustaceans in the wild readily occurs where WSSV is enzootic in cultured penaeids (Lo and Kou 1998, Maeda et al. 1998a, De La Pena et al. 2007, Baumgartner et al. 2009, Diggles 2020c), probably due to horizontal spillover transfer of the infection through wastewater and/or transmission of the virus by vectors (Kanchanaphuam et al. 1998, Biosecurity Australia 2009, Diggles 2017a, 2020c). Once the disease agent is introduced, there are many species which can act as carriers or reservoirs of infection (OIE 2021b, Diggles 2020c), and indeed, data suggests that zooplankton and/or phyto/pico plankton can vector WSSV prior to infection of cultured prawns (Esparza-Leal et al. 2009, Callinan et al. 2013, Mendoza Cano et al. 2014). Mortality rates vary depending on host species, environmental conditions and other variables such as dose and age of the host, however water temperatures above 33°C appear to prevent WSSV replication and can reduce mortalities of prawns exposed to high water temperatures during the early stages of infection (Rahman et al. 2007).

Wild crustaceans usually exhibit sub-clinical infections which can be exacerbated by stress leading to clinical disease upon their capture and holding (Lo et al. 1996). However, mortalities of wild crustaceans may occur whenever WSSV virus is translocated into new regions where crustaceans are naïve to infection. For example, earlier suspicions that wild prawns and crabs with WSSV qPCR Ct values as low as 13.8 were dying in northern Moreton Bay in 2017-18 (Diggles 2020a) were later confirmed in April 2020 when dead carcasses and dying wild prawns and crabs were found with WSSV qPCR Ct values ranging between 11.1-16.4 in canals along the Logan River, confirming that wild prawns and crabs were dying from WSD in waterways where scavenging fishes were excluded by drum filters (Diggles 2020c).

WSD is often considered mainly a disease of cultured prawns (Supamattaya et al. 1998), but other decapod crustaceans can become infected both naturally and experimentally by injection and by per-os exposure (Flegel 2006). These include freshwater crayfish (Family Parastacidae, Family Cambaridae), crabs (Family Portunidae), lobsters (Family Palinuridae, Family Nephropidae), wild penaeid prawns (Family Penaeidae) and freshwater prawns (Family Palaemonidae) (Peng et al. 1998, Wang et al. 1998, Rajendran et al. 1999, Sahul-Hameed et al. 2000, Edgerton 2004, Musthaq et al. 2006, Baumgartner et al. 2009, Meng et al. 2009, Stentiford et al. 2009, Oidtmann and Stentiford 2011, Bateman et al. 2012, Ke et al. 2021). In fact, all decapod crustaceans from marine, brackish and freshwater, as well as planktonic copepods, parasitic copepods that infect fish, barnacles, brine shrimp (*Artemia salina*), rotifers, insect larvae, and polychaetes are considered potential hosts or vectors for WSSV (OIE 2021b). Crabs can be infected *per-os* and die from WSD without showing white spots externally (Sahul-Hameed et al. 2003). White spots may occur inside the carapace of WSSV infected crabs (Raja et al. 2015), however these can also be present in WSSV-negative crabs, probably due to mineral mobilisation within the carapace during the pre-moult stage of the moult cycle (Diggles et al. 2020). Other carrier species can become infected but do not show signs of disease, including rotifers, bivalves, polychaete worms and non-decapod crustaceans

including *Artemia salina*, copepods, and aquatic arthropods such as sea slaters (Isopoda) and insect larvae (Sahul-Hameed et al. 2003, Mendoza Cano et al. 2014). These species can act as mechanical vectors capable of accumulating high concentrations of viable WSSV with persistent, life long infections (OIE 2021b). Although there is no evidence of virus replication within most of these hosts (Stentiford et al. 2009), Overstreet et al. (2009) found evidence of replication of WSSV in the copepod *Ergasilus manicatus* parasitic on fish gills, and Desrina et al. (2013) found infection suggesting replication in polychaetes. Pramod Kiran et al. (2002) investigated vertical transmission in *M. rosenbergii* and found that eggs of experimentally infected brooders were WSSV-negative by cPCR, but the larvae that hatched from them were positive. The OIE (2021b) considers all life stages of crustaceans to be potentially susceptible, from eggs to broodstock. The most likely life stages for detection of WSSV are late post-larval stages, juveniles and adults, while the probability of detection can be increased by exposure to stressful conditions (OIE 2021b) such as rapid reductions in water temperature.

Lobsters in both F. *Palinuridae* and F. *Nephropidae* tend to be more resistant to WSSV infection than prawns. Bateman et al. (2012) found that clinical WSD occurred followed by mortalities of up to 55% within 6 days in European lobsters *Homarus gammarus* (F. *Nephropidae*) after being fed a single 50 mg ration from a *P. vannamei* with clinical WSD (total dose c. 1.82×10^{11} viral copies). Lobsters which were fed lower WSSV doses (between 2.3×10^{6} and 2.58×10^{8} viral copies) were infected but did not become clinically diseased, nevertheless a slightly increased mortality rate (up to 22%) was observed compared to controls (Bateman et al. 2012). Overall 94% of *H. gammarus* fed 50 mg rations of WSSV infected prawn tissues became infected, leading those authors to conclude that the limiting factor in the rapid appearance of WSD in European lobsters was the initial WSSV dose; a low-level infectious dose establishes latent infection, while a high-level dose progresses more rapidly to disease (Bateman et al. 2012).

WSSV has been experimentally or naturally transmitted to at least 7 species of spiny lobsters in the genus *Panulirus* (see Chang et al. 1998a, Wang et al. 1998, Rajendran et al., 1999, Musthaq et al., 2006, Ross et al. 2019a) with variable mortalities. For example, Chang et al. (1998a) successfully infected *Panulirus versicolor* and *Panulirus penicillatus* with WSSV via the *per-os* route by feeding them muscle tissue of WSD infected *P. monodon*, generating WSSV inclusions in the gills, stomach, cuticular epidermis and hepatopancreas, but no mortalities occurred in these species after 20 days. Wang et al. (1998) successfully infected 40-80% of *P. ornatus*, *P. versicolor*, *Panulirus longipes* and *P. penicillatus* with WSSV via the *per-os* route after a single feed of muscle tissue of a WSD infected *P. monodon*, but again no mortalities were observed after 20 days. Rajendran et al. (1999) found WSD mortality rates after 70 days ranged from 33.2% in *Panulirus homarus* injected with WSSV filtrates obtained from *P. monodon* with clinical WSD, to 16.6% in *P. ornatus* and *P. polyphagus* which were infected via the *per-os* route when fed WSSV positive *P. monodon* muscle tissue. The study of Musthaq et al. (2006) found that intramuscular injection with 300µg of haemolymph filtrate isolated from *P. monodon* with clinical WSD resulted in 100% mortality from WSD in both *Panulirus homarus* and *Panulirus ornatus*, at 168 and 120 h, respectively. In contrast, both *P. homarus* and *P. ornatus* became subclinically infected for 4 days but none died when they were exposed to WSSV via the *per-os* route (Musthaq et al. 2006). Based on the results from Bateman et al. (2012), the differences between these various studies in mortality rates after *per-os* exposure to WSSV may simply be due to differences between the dose rates used to infect experimental lobsters. A study by Ross et al. (2019a) found that *Panulirus argus* was highly susceptible to WSSV via intramuscular injection, resulting in WSD and mortalities up to 88% four weeks post inoculation. In the same study it was found that 100% of *P. argus* were also infected with WSSV via

waterborne transmission, but infection was subclinical and WSSV burdens were low after four weeks. As was the case with *P. argus* infected with PaV1, behavioural studies indicated that *P. argus* can detect and avoid conspecifics infected with WSSV, and the avoidance response was strongest for the most heavily infected individuals (Ross et al. 2019a).

It has been reported that WSSV can be inactivated in 20 min at 50 °C (Maeda et al. 1998b), in 1 minute at 60°C and 0.2 min at 70°C (Nakano et al. 1998), though Chang et al. (1998b) stated that 70°C for 5 min was required to completely inactivate the virus. Methodological variations may explain some of these differences, however these authors all examined free virus suspensions isolated from host tissues prior to heat treatment, and they did not examine whether WSSV was protected from heat while *in-situ* inside the tissues of infected hosts. Reddy et al. (2011a, 2011b, 2013) reported that WSSV inside the tissues of *P. monodon* survived in boiling water for up to 30 minutes at 100°C without full inactivation, however when their study was repeated by Aranguren Caro et al. (2020), they found that cooking shrimp tissue at 100°C for >1 minute was sufficient to prevent transmission of WSSV via the *per-os* route.

5.2.5 Release assessment

WSSV is now known to occur in south east QLD in a range of crustacean species within the Moreton Bay White Spot Biosecurity Area, and is likely to remain in this region for the foreseeable future (Diggles 2020c). Transmission of WSSV can occur horizontally through contact with viral particles in the water, however the most effective method of transmission is via the *per-os* route when infected tissue is ingested (Soto and Lotz 2001, Wu et al. 2001, Raja et al. 2015), while the existence of "true" vertical transmission has not been demonstrated (OIE 2021b). This suggests that a major pre-requisite for establishment of WSSV in a new environment is whether the virus can become embedded in populations of crustacean hosts (including plankton) that occur in the lower trophic levels of food chains which, via predation, leads to their eventual ingestion by commercially important wild caught or cultured crustaceans, resulting in WSSV infections that are detectable in fisheries and aquaculture industries (Diggles 2020c). The incursion and subsequent establishment of WSSV in the Moreton Bay White Spot Biosecurity Area has resulted in a need to implement a range of biosecurity requirements to try to prevent the anthropogenic spread of the disease agent from the infected zone into other areas of QLD and Australia (DAF QLD 2017). Some of these requirements include application of strict sanitary measures requiring all prawn products exiting the zone to be either cooked or exposed to 50 kGy of gamma irradiation (Diggles 2020a). These sanitary measures may reduce the risk of WSSV spread via anthropogenic movement of infected prawns from south east QLD, however there is no way of preventing movements of water or natural migration of wild crustaceans out of the Moreton Bay White Spot Biosecurity Area.

The historical absence of WSD on prawn farms along the Logan River, which have been established there for decades, and failure to detect WSSV in various species of prawns sampled from Moreton Bay for surveillance and the University of Queensland Marine Parasitology field course (PA305) from the 1980's till 2006 (Paynter et al. 1985, Spann and Lester 1996, Owens 1997), suggest a recent introduction of WSSV into the Moreton Bay region sometime after 2006 and prior to November 2016 (Diggles 2017a, 2020a, 2020c, Oakey et al. 2019). Indeed, the incursion can be explained by at least one successful recent (post-2006 and pre-December 2016) WSSV introduction via imported prawns used as bait or burley (Kantar Public 2019), followed by a modest founder effect as that strain adapted to local conditions and local hosts (Diggles 2020c). Other potential pathways for recent introduction of WSSV, such as via

ballast water from international shipping at the Port of Brisbane, also cannot be entirely ruled out, although they appear far less likely as the ballast water pathway has never been previously recorded to disseminate WSSV anywhere in the world. A spontaneous recent emergence of a local "endemic strain" of WSSV can be ruled out with high confidence, due to the fact that both the Logan River and Moreton Bay strains of WSSV are of the more recent "shrunken genome" type, which differs significantly from the larger genomes of the ancestral WSSV strains that were collected from their original sites of emergence (Kawato et al. 2019, Oakey et al. 2019, Ki et al. 2021).

Because WSSV was exotic to Australia, the virus has historically been absent from northern Australian waters despite there being suitable conditions for establishment. However, due to the relatively recent introduction of WSSV into south east QLD, it is now possible that the virus could be introduced into north QLD by natural northward movements of wild crustaceans (Ruello 1977, Montgomery 1990). Once introduced in the region, it is possible that WSSV could then be introduced into the hatchery environment via infected broodstock or planktonic crustaceans in intake water, from which larval and juvenile TRL could be exposed to the virus. Taking into account the information above, the risk of release is not negligible, and unrestricted likelihood estimations for the release of WSSV into northern Australian waters via the various release pathways are provided below.

Release assessment for infection with white spot syndrome virus (WSSV)

Pathways	Via broodstock into hatchery in north QLD	Via water into hatchery in north QLD	Via juveniles into waters of QLD	Via juveniles into waters of the NT	Via juveniles into waters of WA
Likelihood of release	Low	Moderate	Low	Low	Low

5.2.6 Exposure assessment

All of the species of decapod crustaceans endemic to northern Australia are likely to be susceptible to WSSV including not only commercially important species such as TRL, but also mud crabs (*Scylla* spp.) and penaeid prawns. This suggests that WSSV released through the various pathways examined here, (particularly in juvenile TRL held at high densities in grow out rafts), could come in contact with many known susceptible species. WSSV remains infective for > 30 days in seawater at 30°C and > 40 days at 25°C under laboratory conditions (Momoyama et al. 1998), but the virus can be inactivated by exposure to various chemicals and UV irradiation (Chang et al. 1998b, Balasubramanian et al. 2006, Oseko et al. 2006). Over the years a range of UV doses have been reported to effectively inactivate WSSV, with different research groups finding nearly 2 orders of magnitude difference in effective dose rates (10-921 mJ/cm^2) (Chang et al. 1998b, Nakano et al. 1998, Balasubramanian et al. 2006, Oseko et al. 2006), possibly due to differences in methodology, initial viral dose studied or the susceptibility of hosts used in bioassays to determine virus viability post-treatment. WSSV viral replication is inhibited at water temperatures above 33°C (Rahman et al. 2007), however, water temperatures are not high enough (>33°C) to prevent establishment of WSSV in vast regions of northern Australia.

Susceptible crustaceans in the wild can also become infected with WSSV via *per-os* exposure through cannibalism (Bateman et al. 2012, OIE 2021b), and indeed the *per-os* route is more effective for establishing WSSV infections than horizontal exposure through the water (Soto and Lotz 2001, Raja et al. 2015). If a susceptible wild or cultured crustacean came in contact with WSSV infected material through the pathways described above, given the high infectivity of WSSV, based on minimum infectious dose

calculations consumption of even a small (<50 mg) portion of a moderately WSSV infected crustacean or vector can result in establishment of a subclinical carrier state (Oidtmann and Stentiford 2011, Bateman et al. 2012). Wild crustaceans often carry sub-clinical WSSV infections (Lo et al. 1996), but they can also experience WSD and die with high viral loads if the virus is introduced into regions where it is not endemic (Diggles 2020c). Thus if the susceptible crustacean in the index case died, cannibalism of dead and moribund crustaceans can be a potent source of WSSV, thereby propagating infection (Soto and Lotz 2001, Soto et al. 2001, Bateman et al. 2012, Raja et al. 2015). Predation of moribund crustaceans by fish may modulate transmission and spread of WSSV in some index cases (Biosecurity Australia 2009, Diggles 2020c), however, crustaceans (particularly crabs and lobsters) are important scavengers in inshore tropical areas which would be equally likely to encounter WSSV infected material.

Common inshore scavenging crabs present in northern Australia such as *Scylla serrata*, *Charybdis* spp. and *Uca* spp. are known WSSV carriers (Diggles 2020c), and these crabs can subsequently infect other susceptible species via cohabitation (Kanchanaphuam et al. 1998). Other smaller crustaceans such as mysids, copepods or planktonic larval stages of decapods are also susceptible to WSSV infection, and indeed plankton may be the main reservoir for WSSV when environmental virus levels are low (Esparza-Leal et al. 2009, Callinan et al. 2013, Mendoza-Cano et al. 2014, Diggles 2017a). Once WSSV becomes embedded in populations of crustacean hosts (including plankton) that occur in the lower trophic levels of food chains, as it has done in south east QLD, this is a major pre-requisite for establishment of WSSV in a new environment which transmits the virus through the food chain and eventually results in WSSV infections that are detectable in fisheries and aquaculture industries (Diggles 2020c). Thus, a large amount of empirical evidence suggests that once WSSV is introduced into a suitable region, due to the wide range of potential host and carrier species in the environment, it is likely to become established in wild populations of crustaceans and/or other carriers (Lo and Kou 1998, Maeda et al. 1998a, Hasson et al. 2006, Baumgartner et al. 2009, Oidtmann and Stentiford 2011, Macias-Rodriguez et al. 2014, Diggles 2020c). The likelihood of establishment in cultured crustaceans is even higher, including the risk of industrial sabotage (Jones 2012). Considering all of these factors, the risk of exposure and establishment is non-negligible, and the overall likelihood of exposure and establishment of WSD in suitable environments in northern Australia is considered to be **High**.

5.2.7 Consequence assessment

In areas where WSSV has been introduced, aquaculture industries based on prawns and other crustaceans (e.g. crayfish) have suffered significant production and economic losses (Stentiford et al. 2012). Even if adaptation to the disease agent occurs over time, the presence of the virus represents a significant obstacle to industry competitiveness and profitability. Production in many WSD affected countries overseas eventually recovered, however much of the recovery was due to switching to the faster growing *Penaeus vannamei* (see Flegel 2006, Stentiford et al. 2012), a species which is exotic to Australia and hence this recovery option is not available in this country. There are no commercially available methods of control of WSD (vaccines etc.), besides filtering water and covering of production ponds with mosquito netting (Thitamadee et al. 2016). Under Australian economic conditions, the required changes to prawn farming infrastructure and husbandry practices (filtration of water, lining of ponds, carrier and vector exclusion, minimal/zero water exchange production cycles, development of SPF or SPR prawns lines, see Lightner 2005) impart additional production costs that may reduce industry profitability, at least in the short term. The reduced profitability could discourage investment in crustacean farming in Australia, potentially posing a risk to Australia's future food security (Stentiford 2012, Stentiford et al. 2012). The likely

impacts of introduction of WSD on crustacean aquaculture industries in northern Australia are therefore considered to be extreme.

Infections of wild crustaceans are generally sub-clinical (Lo et al. 1996), and adverse impacts at the population level have not been reported in wild crustaceans in areas where WSSV has been introduced (Maeda et al.1998a, De La Pena et al. 2007, Baumgartner et al. 2009, Flegel 2009). However, the virus can cause disease and mortalities of naïve wild crustaceans (Diggles 2020a, 2020c). Because sub-clinical WSSV infections can revert to the disease state in susceptible species after periods of stress (Lo et al. 1996), this suggests that populations of wild crustaceans adversely affected by environmental stressors (e.g. adverse environmental conditions, rapid drops in water temperature or exposure to pollutants such as pesticides and herbicides) may also experience reduced resilience or "silent mortalities" (Behringer et al. 2012a, Stentiford et al. 2012, Shields 2012) due to WSSV infection, as has been reported for some other viral pathogens of prawns (Couch and Courtney (1977). Any adverse effects could result in ecological harm to aquatic environments, potentially resulting in significant cultural and socio-economic harm to regional communities in northern Australia and elsewhere in the country.

Furthermore, as WSSV is a listed disease agent notifiable to the OIE and NACA, significant trade implications would follow its translocation. Indeed, establishment of WSD in a new region of northern Australia would require intervention by government authorities and disruption to normal trade in crustacean commodities by commercial fisheries and crustacean gathering by recreational fishers if attempts were made to limit its potential spread into uninfected areas. If the introduction of WSD is detected early enough in a confined population of hosts, there is a chance that eradication can be achieved (Biosecurity Australia 2009), although eradication programs are expensive processes, especially if a subsequent declaration of freedom from the disease is required, as extensive surveys would need to be undertaken (East et al. 2004, 2005). Taking all of these factors into consideration, even though the environmental impacts of introduction of WSD are difficult to determine, economic impacts on domestic and international trade are both considered to be high, while the impact on crustacean aquaculture industries would be extreme and irreversible, hence the consequences of introduction and establishment of WSD into the environment of northern Australia via the identified risk pathways are likely to be **High.**

5.2.8 Risk estimation

The unrestricted risk associated with infection with WSSV is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The unrestricted risk estimate for infection with WSSV exceeds the ALOP for all pathways, suggesting that additional risk management is required for this disease agent.

Risk estimate for infection with white spot syndrome virus (WSSV)

Pathways	Via broodstock into hatchery in north QLD	Via water into hatchery in north QLD	Via juveniles into waters of QLD	Via juveniles into waters of the NT	Via juveniles into waters of WA
Combined likelihood of release and exposure	Low	Moderate	Low	Low	Low
Consequences of establishment	High	High	High	High	High
Risk estimation	Moderate risk 12	High risk 16	Moderate risk 12	Moderate risk 12	Moderate risk 12

5.3 Milky haemolymph disease of spiny lobsters (MHD-SL)

5.3.1 Aetiologic agent: An undescribed gram-negative intracellular rickettsia like organism (RLO) with affinities with the alpha-proteobacteria, that infects lobsters. Although the pathology of RLO diseases in crustaceans appears similar in a wide range of hosts, they are genetically distinct disease agents which may not necessarily be closely related in different host taxa (Nunan et al. 2010).

5.3.2 Under official control in Australia: QLD, WA **Zoonotic:** No

5.3.3 Australias status: The agent responsible for MHD-SL has never been recorded in Australia and is considered exotic. However, various RLOs are known to infect crustaceans in Australia, including *Coxiella cheraxi* which was associated with mortalities in *Cherax quadricarinatus* cultured in north Queensland (Tan and Owens 2000, Edgerton et al. 2002), and an undescribed RLO infecting wild caught sand crabs (*Portunus pelagicus*) in the NT (Diggles et al. 2013).

5.3.4 Epizootiology

Rickettsia like organisms (RLOs) are intracellular prokaryotes that have been reported from a wide range of crustaceans (Brock et al. 1986, Brock and Lightner 1990, Bower et al. 1994, 1996, Krol et al. 1991). Most RLO infections of crustaceans are not considered significant, however occasionally there are reports of serious disease outbreaks associated with RLOs in cultured crustaceans including freshwater crayfish (Tan and Owens 2000), penaeid prawns (Nunan et al. 2003a, 2003b), crabs (Eddy et al. 2007) and also lobsters (OIE 2008, Nunan et al. 2010). For example, in 1999 a new RLO was associated with milky haemolymph and severe mortalities of prawns *Penaeus monodon* farmed in grow-out ponds in Madagascar (Nunan et al. 2003a). In experimental trials the RLO involved was able to infect *P. vannamei*, but only if it was injected, with infection unable to be achieved by the oral route (Nunan et al. 2003b). The failure to transfer infection via the oral route was considered significant, as it suggests that this particular agent may only cause disease in grow out ponds when hosts are compromised or stressed, or else it could suggest that a parasite or other aquatic species may be required to complete the infection process (Nunan et al. 2003b).

Then in late 2006, a "milky haemolymph disease" emerged in spiny lobsters (*Panulirus versicolor*, *P. ornatus*, *P. homarus*, and *P. polyphagous*) cultured in Vietnam (Diggles 2008, OIE 2008, Hung and Tuan 2009, Callinan and Corsin 2009). One of the first reports of this disease was in spiny lobsters collected from the Bay of Lang Co in central Vietnam and held in a nearby onshore holding system (Andrew Jeffs, personal communication, Diggles 2008). The milky haemolymph disease of spiny lobsters (MHD-SL) was so named because the haemolymph of affected lobsters became turbid and turned 'milky' in appearance in severely infected individuals (Nunan et al. 2010). The aetiological agent involved was a novel rod shaped (0.6 µm x 1.4 to 2.0 µm), gram negative bacteria which occurred in large numbers in haemolymph and muscle, but which remained unculturable using conventional microbiological media, and hence displayed many RLO characteristics (Diggles 2008, OIE 2008, Lightner et al. 2008, Nunan et al. 2010). The disease outbreak caused large scale mortalities in Vietnam in 2007 resulting in a significant drop in lobster aquaculture production from 1900 to 1400 metric tonnes (Hung and Tuan 2009, Callinan and Corsin 2009, Petersen and Phuong 2011). MHD-SL subsequently re-emerged in Vietnam in early

2012, causing a substantial decline in production over subsequent months, although not to the same extent seen in 2008/09 when production was halved (Jones et al. 2015).

Onset of milky haemolymph disease was reportedly very rapid, with affected lobsters initially becoming inactive and ceasing to feed (OIE 2008). Within another 3-5 days affected lobsters were observed with milky haemolymph under swollen abdominal pleura of the exoskeleton (visible on ventral side), with mortalities beginning soon after clinical signs became apparent (OIE 2008). The course of disease within a single cage was usually protracted, with 2-5 animals dying per day, so that it took up to 2 months for the entire cage population to be lost (Callinan and Corsin 2009). Haemolymph drawn with a syringe from affected lobsters did not clot and ranged from slightly cloudy or turbid to milky white (OIE 2008, Nunan et al. 2010). Dissection of affected lobsters demonstrated the presence of milky coloured haemolymph in the haemocoel and tissue spaces and white hypertrophied connective tissues (especially serosa and capsules) of all major organs and tissues (OIE 2008, Nunan et al. 2010). The destruction of circulating hemocytes resulted in haemolymph that did not clot, which in conjunction with injuries from handling and confinement together with organ dysfunction was likely to contribute to morbidity and mortality (Nunan et al. 2010).

The disease was problematic only in net-pen-reared spiny lobsters which were being fed a variety of fresh foods including trash fish, molluscs and decapod crustaceans acquired locally from fishers (OIE 2008). Overall mortality rates of between 20 and 30% were being observed during the grow out cycle, with highest mortality rates in net pens that sat on the bottom substrate (Callinan and Corsin 2009, Hung and Tuan 2009). However, not all mortalities in Vietnam at that time were caused by MHD-SL, due to the presence of other disease syndromes including “black gill” caused by *Fusarium* spp., as well as “red body” caused by vibriosis (Callinan and Corsin 2009). Furthermore, some of the affected lobsters were also apparently infected by an undescribed virus, as evidenced by the presence of many conspicuous enlarged nuclei with marginated chromatin and cowdry-like intranuclear inclusion bodies in haemocytes, the epithelium of the digestive gland and subcuticular epithelium of the gills (Diggles 2008). In any case, because of the significant reduction in production, MHD-SL was temporarily listed in the OIE Aquatic Animal Health Code (OIE 2009) as ‘under study’ for possible listing as a notifiable disease of *Panulirus* spp., and an OIE disease card was developed to help clarify the case definition (see OIE 2008, 2009, Nunan et al. 2010).

Subsequent research undertaken in Vietnam found that while the disease could be experimentally transmitted from diseased to healthy lobsters by cohabitation and by injection of unfiltered haemolymph (OIE 2008), outbreaks of MHD-SL were strongly associated with poor water quality and poor husbandry following bacterial decomposition of trash fish feeds (OIE 2008, Nunan et al. 2010, Petersen and Phuong 2011). The disease remained problematic only in net-pen-reared spiny lobsters which were being fed a trash fish, mollusc and/or decapod crustacean diet acquired locally from fishers (OIE 2008). Improved husbandry reduced the impact of the disease, and in view of this MHD-SL was not listed by the OIE as a notifiable disease as it was considered mainly due to opportunistic invasion of hosts which were compromised by adverse rearing conditions, which duly lead to a greater research focus on development of formulated (“artificial”) feeds (Hung et al. 2010, Petersen and Phuong 2011, Perera and Simon 2015, Marchese et al. 2019).

Similar RLOs have more recently been reported causing clinical disease including milky haemolymph, during investigations into mortalities of up to 100% in spiny lobsters (*Panulirus longipes* and *P. homarus*) cultured in seacages in Indonesia (Nur and Yusnaini 2018, Sudewi et al. 2018a, 2018b, 2020). However, as in Vietnam, the mortalities of cage cultured TRL in Indonesia were associated with a range of disease syndromes, including black gill caused by *Fusarium* spp. and vibriosis, as well as ubiquitous commensals such as *Octolasmis* spp. (see Sudewi et al. 2018a, Nur and Yusnaini 2018). Milky disease was reported almost year-round in Lombok from 2012 to 2016, and in Pegametan Bay, North Bali in 2016, but only in farmed lobsters, and not in wild lobsters (Sudewi et al. 2020). Sudewi et al. (2018b) reported that MHD-SL was experimentally transmitted to healthy *P. homarus* by injection (resulting in 100% mortality within 15 days) and horizontally via the water (resulting in 50% mortality within 7 days). In contrast, no mortalities occurred when lobsters were exposed to the RLO *per-os* via the oral route after being fed frozen (-20°C) heavily infected lobster muscle tissue, however subclinical infections were initiated and detected by cPCR (Sudewi et al. 2018b). Furthermore, one lobster exposed via the oral route showed clinical signs of milky haemolymph disease, but it recovered once it ceased feeding on infected tissues (Sudewi et al. 2018b). Genetic analysis confirmed that the RLO agent infecting TRL in Indonesia exhibited 99% nucleotide sequence identity with the RLO responsible for MHD-SL in Vietnam (Sudewi et al. 2020).

It is known that several RLOs infect crustaceans in Australia. For example, during the summer of 1990 heavy mortalities were reported in redclaw crayfish (*Cherax quadricarinatus*) cultured in grow out ponds on a farm in north Queensland (Ketterer et al. 1992). Over the next few years similar RLOs were occasionally found associated with disease in cultured redclaw (Edgerton and Prior 1999, Edgerton et al. 2002) and eventually *Coxiella cheraxi* was described by Tan and Owens (2000). Experiments confirmed that *C. cheraxi* was highly pathogenic for freshwater crayfish, resulting in 100% mortality when injected into healthy crayfish at 28°C (Tan and Owens 2000). Horizontal transmission was also confirmed via the water (30% mortality within 4 weeks) and *per-os* with food (10% mortality over a 4 week period) (Tan and Owens 2000). Over the past 30 years *C. cheraxi* has continued to cause mortalities and has proven to be an important pathogen of crayfish cultured in northern Australia (Elliman and Owens 2020).

More recently an undescribed RLO was reported to infect wild caught blue swimmer crabs (*Portunus pelagicus*) in the NT (Diggles et al. 2013). The overall prevalence of the RLO infection in blue swimmer crabs from all sites detected using histopathology was low (4.4%, n = 90 crabs), but there appeared to be some evidence of seasonal and spatial variation (Diggles et al. 2013). Spatially, prevalence was highest at 10.5% (n = 19) at Outer Darwin Harbour, followed by 3.3% at Bynoe Harbour (Diggles et al. 2013). Histology demonstrated large numbers of RLO organisms inside intracytoplasmic inclusions in hypertrophied digestive gland epithelial cells (Diggles et al. 2013). Field data revealed most of these affected sand crabs were lethargic and weaker than unaffected crabs at capture, but no other gross signs of disease were observed, the colour of the haemolymph of the affected crabs was normal, and retained its ability to clot (Diggles et al. 2013). Exclusion testing performed at the Australian Animal Health Laboratory (AAHL) found that the rickettsia-like organisms did not react with PCR designed for MHD-SL, nor did they react with PCR designed to detect rickettsia-like organisms from the European shore crab or *Penaeus monodon* (see Diggles et al. 2013).

5.3.5 Release assessment

The agent responsible for MHD-SL has never been recorded in Australia and is considered exotic. However, the disease status of TRL populations in northern Australia is poorly known. Because of this there is a high likelihood that new (currently undescribed) bacterial diseases could emerge at some stage in the future during development of the TRL industry in northern Australia in a scenario similar to the emergence of MHD-SL in Vietnam. Furthermore, various endemic RLOs are known to infect wild and cultured crustaceans in Australia, and some of these are known to cause disease and mortality of their hosts (Ketterer et al. 1992, Tan and Owens 2000, Edgerton et al. 2002, Diggles et al. 2013). Translocation of live crustaceans is known to pose a high risk of introduction and establishment of RLOs, as evidenced by the appearance of apparently Australian-endemic RLOs in redclaw crayfish translocated into central America for the purposes of aquaculture development (Romero et al. 2000). Under situations where horizontal infection by the waterborne route occurs, it is thought that the gills are likely to be an important site of infection (Tan and Owens 2000). While some species of RLO can be transmitted via the *per-os* route (including the agent responsible for MHD-SL, see OIE 2008, Sudewei et al. 2018b), others may not be readily transmissible in this manner (Nunan et al. 2003b). The RLO agent responsible for MHD-SL can cause systemic infections of all major organ systems in lobsters, including the gonad (Sudewei et al. 2018b), however the existence of "true" vertical transmission of MHD-SL between generations has not been demonstrated. Taking into account the information above, the risk of release is not negligible, and unrestricted likelihood estimations for the release of milky haemolymph disease of spiny lobsters (or a similar endemic RLO) into northern Australian waters via the various release pathways are provided below.

Release assessment for Milky haemolymph disease of spiny lobsters (MHD-SL)

Pathways	Via broodstock into hatchery in north QLD	Via water into hatchery in north QLD	Via juveniles into waters of QLD	Via juveniles into waters of the NT	Via juveniles into waters of WA
Likelihood of release	Moderate	Moderate	Moderate	Moderate	Moderate

5.3.6 Exposure assessment

Wild and cultured crustaceans throughout the marine environment in northern Australia are already at risk of natural exposure to endemic RLOs. However, infection and establishment of RLOs in new hosts or locations would occur only if viable RLOs were introduced into an area where susceptible hosts were present under suitable environmental conditions for transmission. Environmental conditions throughout NA are likely to be suitable for establishment of MHD-SL as well as similar endemic RLOs. The RLO responsible for MHD-SL can be transmitted horizontally by co-habitation or the *per-os* routes (OIE 2008, Sudewei et al. 2018b), however the minimum infective dose for either pathway has not been determined. While they are obligate intracellular bacteria, RLOs may survive for extended time periods in the water column (Tan and Owens 2000). Furthermore, it is known that some RLOs can survive freezing, although their infectivity upon thawing may be reduced for some routes of exposure such as *per-os* (Nunan et al. 2003b). The ability of RLOs to survive outside the host cell for long time periods may be why horizontal transmission of RLOs via the water is successful, at least within confined tanks or over relatively short distances of 10s of meters during outbreaks of MHD-SL in caged TRL. The transmission and spread of

these disease agents in populations of wild crustaceans after an index case occurs may be modulated somewhat by predation of moribund crustaceans by non-susceptible species such as finfish, although crustaceans (particularly crabs and lobsters) are important scavengers in inshore tropical areas which would be equally likely to encounter RLO infected material,

Lobsters that survive infection with RLOs may become life-long subclinical carriers of the disease agent (Sudewei et al. 2018b), however the potential for vertical transmission is unknown. Nevertheless, early settlement post-larvae (puerulus) are known to be susceptible to RLO infection, presumably via the waterborne route, and RLOs may survive free in seawater for long periods, possibly up to 15 days or more (Tan and Owens 2000). This means that without appropriate biosecurity, waterborne transmission of RLOs from broodstock to puerulus and juvenile lobsters would appear likely within a confined hatchery situation. The RLO responsible for MHD-SL appears to have low host specificity and can infect many species of TRL, hence if infected juvenile TRL were translocated into new regions, native populations of TRL in northern Australia would be vulnerable to infection. On the other hand, these disease agents do not generally appear to cause disease unless their hosts are stressed and/or held in confinement at high densities, which may not occur in the natural environment, but is certain to occur if juvenile TRL are confined in sea rafts during grow out. Given that RLOs could occur in lobster broodstock and these disease agents can be horizontally transmitted within hatchery environments in the absence of appropriate biosecurity precautions, and environmental conditions are likely to be suitable for disease transmission in the wild in northern Australia, the risk of exposure and establishment is non-negligible, and the overall likelihood of exposure and establishment of milky haemolymph disease of spiny lobsters (or a similar endemic RLO) is considered to be **Moderate**.

5.3.7 Consequence assessment

Although some types of RLO are already present in populations of wild and cultured crustaceans in some regions of Australia, it is possible that other regions remain free of infection at this time. There is certainly evidence that once established within populations of TRL confined in net pens, the agent responsible for MHD-SL and other similar RLOs can cause major disease outbreaks and significant impacts on populations of cultured TRL (OIE 2008, Callinan and Corsin 2009, Nunan et al. 2010, Sudewi et al. 2018a). However, the disease does not appear to affect wild populations of TRL (OIE 2008, Sudewi et al. 2020). In Vietnam the disease remained problematic only in net-pen-reared spiny lobsters which were being fed a trash fish, mollusc and/or decapod crustacean diet acquired locally from fishers (OIE 2008). Improved husbandry reduced the impact of the disease, and in view of this MHD-SL was not listed by the OIE as a notifiable disease as it was considered mainly due to opportunistic invasion of hosts which were compromised by adverse rearing conditions. Nevertheless, MHD-SL remains listed as a reportable disease in QLD and WA (Table 2), and hence the spread of MHD-SL (or a similar endemic RLO) to new areas may require intervention by government authorities and disruption to normal trade in crustacean commodities by commercial fisheries and crustacean gathering by recreational fishers if attempts were made to limit its potential spread into uninfected areas.

Taking all of these factors into consideration, the establishment of MHD-SL or other RLOs into new areas would have moderate consequences for crustacean aquaculture, but would be unlikely to cause any noticeable environmental effects. Whilst emergence of the disease could cause economic harm and pose an obstacle to future investment in TRL aquaculture in NA, it may be amenable to control by improvement of husbandry related factors in grow out rafts and use of formulated diets. For these reasons, the consequences of introduction and establishment of milky haemolymph disease of spiny lobsters (or a

similar endemic RLO) into the environment of northern Australia via the identified risk pathways would likely be **Low**.

5.3.8 Risk estimation

The unrestricted risk associated with milky haemolymph disease of spiny lobsters (or a similar endemic RLO) is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The unrestricted risk estimate for milky haemolymph disease of spiny lobsters (or a similar endemic RLO) exceeds the ALOP for some pathways, suggesting that additional risk management is required for these disease agents.

Risk estimate for Milky haemolymph disease of spiny lobsters (MHD-SL)

Pathways	Via broodstock into hatchery in north QLD	Via water into hatchery in north QLD	Via juveniles into waters of QLD	Via juveniles into waters of the NT	Via juveniles into waters of WA
Combined likelihood of release and exposure	Moderate	Moderate	Moderate	Moderate	Moderate
Consequences of establishment	Low	Low	Low	Low	Low
Risk estimation	Low risk 8	Low risk 8	Low risk 8	Low risk 8	Low risk 8

5.4 Microsporidosis

5.4.1 Aetiologic agent: Microsporidians are obligate intracellular parasites known to infect a wide variety of eukaryotic hosts, including fish, mammals, and arthropods including crustaceans (Lom and Dykova 1992, Stentiford et al. 2016).

5.4.2 Under official control in Australia: WA, ACT **Zoonotic:** Potentially

5.4.3 Australias status: A range of microsporidians are known to occur in a wide variety of crustacean host species in Australia, including penaeid prawns (*Penaeus monodon*, *Penaeus esculentus*, *Penaeus semisulcatus*, *Penaeus merguiensis*, *Melicertus latisulcatus*, *Penaeus* spp.), freshwater prawns (*Macrobrachium* spp.), freshwater crayfish (*Cherax destructor*, *Cherax quadricarinatus*, *Cherax tenuimanus*, *C. cainii*, *Cherax* spp.), crabs (*Portunus pelagicus*) and lobsters (*Panulirus* spp.) (Dennis and Munday 1994, O'Donoghue and Adlard 2000).

5.4.4 Epizootiology

Microsporidians are common parasites of crustaceans, with a large number of genera being reported from crustacean hosts all over the world, including *Agmasoma*, *Ameson*, *Enterocytozoon*, *Enterospora*, *Flabelliforma*, *Glugoides*, *Hepatospora*, *Indosporus*, *Myospora*, *Nadelspora*, *Nosema*, *Ordospora*, *Pleistophora*, *Thelohania*, *Vavraia*, *Tuzetia* and others (O'Donoghue and Adlard 2000, Stentiford et al. 2007, 2010, Bateman et al. 2016). Microsporidosis generally occurs at low prevalence in wild crustaceans (Owens and Glazebrook 1988), and occasionally they are reported in cultured crustaceans as well (Anderson et al. 1989, Flegel et al. 1992, Hudson et al. 2001, Vidal–Martinez et al. 2002, Tourtip et al. 2009, Chaijarasphong et al. 2021). Crustaceans infected by microsporidians often have opaque musculature (reminiscent of chalky or "cooked meat") and are unmarketable, whilst infections have also been associated with significant disease and mortality of cultured crustaceans in some instances (Flegel et al. 1992, Lightner 1996b, Hudson et al. 2001, Stentiford et al. 2018).

The life-cycles of many microsporidians affecting crustaceans may be indirect (Breed and Olson 1977, Flegel et al. 1992, Herbert 1988, Edgerton et al. 2002, Stentiford et al. 2018), which means that the chances of transmission of the disease in some crustacean populations may be reduced as it may depend on the presence of intermediate hosts (such as copepods, insects or finfish). However, there may be some exceptions to this (Langdon and Thorne 1992, Hudson et al. 2001, Tang et al. 2016, Salachan et al. 2017, Chaijarasphong et al. 2021), and because the life cycles of microsporidians that infect crustaceans are so poorly understood, the possibility remains that life cycles may differ even between closely related species (Edgerton et al. 2002). For example, *Enterocytozoon hepatopenaei* (EHP), which has spread and caused significant economic losses in many prawn farming countries throughout Asia in recent years (Sritunyalucksana et al. 2014, Tang et al. 2015, 2016, Biju et al. 2016, Otta et al. 2016, Rajendran et al. 2016, Han et al. 2016, Kesavan et al. 2016, Aranguren et al. 2017), is listed by the OIE as an internationally notifiable disease agent of crustaceans (Table 1). Experiments have shown that EHP stages in infected hepatopancreas or voided through the faeces are infective to other prawns via the *per-os* route and by cohabitation without the need for intermediate hosts or other vectors, while in production ponds, EHP is readily transmitted horizontally among prawns through cannibalism or cohabitation (Tang et al. 2016, Salachan et al. 2017, Chaijarasphong et al. 2021). Even so, Salachan et al. (2017) noted that exposure to purified EHP spores failed to induce infections by bath exposure, when added to prawn feed

or when administered by reverse gavage. This could suggest that some examples of direct transmission via *per-os* routes in EHP may be due to exposure to and/or consumption of presporogenic stages, rather than through direct exposure to the spores themselves (Langdon and Thorne 1992). While EHP has not been recorded from Australia and is considered exotic, the identity of a microsporidian infecting the hepatopancreas causing higher than normal mortalities in 7 day old post larvae of *Penaeus japonicus* in a hatchery in Queensland in 1997 was not determined at the time (Hudson et al. 2001). However, given the parasite of *P. japonicus* was associated with significant mortalities, while *E. hepatopenaei* infections alone tend not to cause mortality in infected prawns (Santhoshkumar et al. 2016, Tang et al. 2016, Aranguren et al. 2017), it appears unlikely to be *E. hepatopenaei*, and the identity of this presumably endemic parasite of *P. japonicus* remains to be elucidated. A microsporidian which was detected incidentally at low prevalence in diseased jelly prawns (*Acetes sibogae australis*) infected by a novel haplosporidian in northern Moreton Bay, QLD was taxonomically distinct from *E. hepatopenaei*, providing further evidence EHP is not present in Australia at this time (Diggles 2020a, Diggles et al. submitted).

Many other species of endemic microsporidians have been identified in native crustaceans from a wide range of environments throughout Australia, however the full extent of the distributions of the various parasite species remains largely unknown. Some examples include *Vavraia parastacida* (see Langdon 1991, Langdon and Thorne 1992), *Thelohania* spp. (see Herbert 1988, Shields 1992, Jones and Lawrence 2001), *Thelohania montirivulorum* (see Moodie et al. 2003a), *T. parastaci* (see Jones and Lawrence 2001, Moodie et al. 2003c), *Vairimorpha cheracis* (see Moodie et al. 2003b) and others from wild and cultured freshwater crayfish. The prevalence of microsporidians is often <5% in aquacultured freshwater crayfish (Jones and Lawrence 2001), but can be equally high or higher in wild populations. For example, the prevalence of *Thelohania* spp. in wild yabbies (*Cherax destructor*) in southern Australia can be as high as 38% in some locations (Jones and Lawrence 2001, Moodie et al. 2003c), while in north QLD Herbert (1988) found the prevalence of *Thelohania* spp. in wild *C. quadricarinatus* sampled from the Mitchell River was 7.8%. In contrast, the prevalence of infection of *Ameson* spp. in wild penaeid prawns tends to be quite low, occurring in 0.1% of prawns from northern Australia (Owens and Glazebrook 1988), while *Ameson* spp. was found at similarly low prevalences in wild caught sand crabs (*Portunus armatus*) from Moreton Bay (Shields and Wood 1993).

Microsporidians infecting spiny lobsters also appear to occur at relatively low prevalences. For example, Kiryu et al. (2009) and Small et al. (2019b) reported that microsporidiosis due to *Ameson herrnkindi* is rarely identified in Caribbean spiny lobsters, with only a handful (<10 individuals) of *P. argus* being reported over the previous 30 years, despite the fact that clinical infections are easily detected due to the tail muscle of infected lobsters having conspicuous chalky white "cotton-like" or "cooked flesh" appearance. A pathological survey of wild *P. argus* captured from near St Kitts in the West Indies by Atherley et al. (2020) found the prevalence of *A. herrnkindi* infections was 0.6%, with spores of the parasite being found not only in tail muscle, but also within cardiac muscle, gonad interstitial tissue, antennal gland, hepatopancreas, and hemolymph. Similarly, Itoh et al. (2020) reported wild Japanese spiny lobsters (*Panulirus japonicus*) captured from mid-western Japan were sometimes infected with a new microsporidian they described as *Ameson iseebi*. Again, infected lobsters were conspicuous and displayed tail muscle with a "cotton-like" or "cooked flesh" appearance. The results from the Caribbean and Japan are, therefore, similar to the situation in Australia where Dennis and Munday (1994) reported infections of wild caught *P. ornatus* from Torres Strait and western rock lobsters (*Panulirus cygnus*) from

WA with a microsporidian species morphologically consistent with the genus *Ameson*. The parasite infected tail muscle resulting in a "cooked flesh" appearance at a frequency reported by commercial fishers to be in the order of between 1/1000 and 1/3000 of lobsters handled during processing (prevalence 0.03-0.1%). Little else is known about this parasite and its specific identity and life cycle remain to be determined. Microsporidians are also known to occur in clawed lobsters (Family *Nephrophidae*), as shown by the discovery of *Myospora metanephrops* causing muscle opacity and unusual colouration in scampi (*Metanephrops challengeri*) from New Zealand (Stentiford et al. 2010).

Several of the microsporidian parasites infecting crustaceans are closely related to known pathogens of humans. For example, EHP and the microsporidian in jelly prawns are both phylogenetically placed within the Family *Enterocytozoonidae* (see Diggles et al. submitted), and as such is closely related to *Enterocytozoon bieneusi* which infects the intestinal epithelium causing diarrhoea and disease not only in humans, but also pigs and a range of other mammals and birds (Snowden 2004, Mathis et al. 2005). The close relationship between EHP and *E. bieneusi* raises interesting questions about their evolutionary relationships and the potential role of invertebrates as a source of zoonotic infections (Snowden 2004, Stentiford et al. 2011, Stentiford et al. 2016). Indeed, the potential susceptibility of humans to infection by microsporidians across the phylum appears to be significant, and as such they should be considered potentially zoonotic disease agents (Stentiford et al. 2016).

5.4.5 Release assessment

In Australia, microsporidian infections have been recorded throughout the country in a wide variety of crustacean species, including TRL (Owens and Glazebrook 1988, Dennis and Munday 1994, O'Donoghue and Adlard 2000, Hudson et al. 2001). These parasites appear to occur in a range of environments throughout the country, however the full extent of the distributions of the various species of microsporidian parasites found in crustaceans remains largely unknown. Microsporidians are known to have been translocated into new regions with anthropogenic movements of their hosts. For example, *Hepatospora eriocheir*, another member of the Family *Enterocytozoonidae*, infects the hepatopancreas of the Chinese mitten crab (*Eriocheir sinensis*) in China in 2007 (Wang and Chen 2007), where it causes significant disease in cultured *E. sinensis* (see Ding et al. 2016). However, the same parasite was also found at prevalences of around 70% in introduced populations of *E. sinensis* sampled from the Thames River in the UK (Stentiford et al. 2011). It is thought that *E. sinensis* was introduced into Western Europe in the early 20th century, most likely via a ballast water introduction, with the microsporidian most likely being introduced at the same time (Stentiford et al. 2011). Jones and Lawrence (2001) noted that the emergence of *Thelohania* spp. in cultured yabby (*C. destructor*) populations in Western Australia was probably due to illegal importation of yabbies from the eastern states. It is also possible that microsporidians infecting crustaceans could be translocated in the sea chests of international shipping (Coutts and Dodgshun 2007), and infected crustaceans can also naturally colonise floating vectors such as anthropogenic flotsam, discarded fishing gear and other debris which can make landfall into northern Australia (Wilcox et al. 2013, Heersink et al. 2014, Diggles 2017b).

The likelihood of release will depend on the lifecycle of these parasites and the ability of microsporidian infective stages to remain viable, and it appears that microsporidian spores can remain viable in the natural environment for months to years. For example, spores of *Loma salmonae* remained viable when stored in freshwater or seawater at 4°C for up to 95 days (Shaw et al. 2000), and spores of *Glugea*

stephani remained viable after 17 months at 5°C (Amigo et al. 1996). Indeed, microsporidian spores are known to be extremely robust and some species can also survive and remain viable after long periods of freezing. For example, Overstreet and Whatley (1975) found that spores of *Ameson michaelis* from blue crabs survived 67 days freezing at -22°C. Infectivity of *Nosema apis* spores in bees held at -20°C for 24 hours was not significantly different from that of fresh spores (Bailey 1972), while spores of 31 microsporidian species in water held in liquid nitrogen for 2–25 years remained infective for insect hosts (Maddox and Solter 1996). However, in contrast, Li and Fayer (2006) found the spores of 3 species of *Encephalitozoon* were 100% inactivated by storing them in buffered culture medium at -20°C for between 2 hours (*E. cuniculi*), and 24 hours (*E. intestinalis* and *E. hellem*). So sensitivity to freezing for microsporidian spores is not universal and may vary even between closely related species.

It is known that wild caught TRL in northern Australia can be infected by a microsporidian species morphologically consistent with the genus *Ameson* (see Dennis and Munday 1994), however prevalence of infection is thought to be very low based on the fact that clinically infected lobsters display prominent clinical signs of disease (namely a "cooked flesh" appearance of the tail muscle). Nevertheless, both Small et al. (2019b) and Atherley et al. (2020) noted that the actual prevalence of *Ameson herrnkindi* in *P. argus* in the Caribbean is likely to be under-reported, because subclinical infections are not apparent and would not be noticed or reported by fishers. The same can be said for the situation in Australian TRL. Furthermore, little else is known about the *Ameson* sp. in Australian TRL, including whether it has a direct lifecycle, whilst the potential for different species of *Ameson* to occur on the east and west coasts of Australia cannot be ruled out at the present time. Due to the fact that life cycles of microsporidians may differ even between closely related species (Edgerton et al. 2002), and because of the paucity of knowledge regarding the health status of TRL in Australia, it will be assumed here that *Ameson* spp. in TRL can be transmitted horizontally and directly via ingestion of presporogenic stages, and that more than one species of microsporidian may occur in TRL in northern Australia. Taking into account the information above, the risk of release is not negligible, and unrestricted likelihood estimations for the release of microsporidians into northern Australian waters via the various release pathways are provided below.

Release assessment for Microsporidosis

Pathways	Via broodstock into hatchery in north QLD	Via water into hatchery in north QLD	Via juveniles into waters of QLD	Via juveniles into waters of the NT	Via juveniles into waters of WA
Likelihood of release	Very low	Moderate	Low	Low	Low

5.4.6 Exposure assessment

Wild and cultured crustaceans throughout the marine environment in northern Australia are already at risk of natural exposure to infective stages of microsporidians. Due to the apparent low prevalence of microsporidian infections in adult TRL in Australia, translocation of small numbers of broodstock TRL into hatcheries would appear to represent a very low risk. However, translocation of large numbers of cultured juvenile TRL would increase the risk of exposure of wild crustaceans in northern Australia. The spores of microsporidians are resistant and known to be able to persist in the environment for long periods, extending their period of infectivity. If susceptible species of wild lobsters or other susceptible hosts in northern Australia were exposed to viable infective stages of microsporidians via one of the

identified pathways, infection may occur horizontally via cohabitation or *per-os* if sufficient quantities of infective stages (i.e. an infective dose) were introduced into an area where susceptible hosts were present under conditions suitable for transmission.

Some microsporidians infecting crustaceans are transmitted directly (Langdon and Thorne 1992), but the minimum infective dose of infective stages required for successful transmission has not been determined for the majority of species, and this also probably will vary depending on the identity of the host and its immune status. Infection can be achieved by the *per-os* route for at least some microsporidians if susceptible crustaceans ingest presporogenic stages, though susceptibility may vary between hosts (Langdon and Thorne 1992). When the natural course of infection is considered, it is clear that infection can be theoretically achieved after exposure to a dose as small as a single viable spore or presporogenic stage, though the dose required to cause host mortality will depend on many factors. Lightly infected (sub-clinical) lobsters may contain thousands of spores and presporogenic stages, while heavily infected lobsters may contain millions of spores and presporogenic stages (Lom and Dykova 1992, Small et al. 2019b, Atherley et al. 2020, Itoh et al. 2020).

Given the spores of microsporidians can remain viable in the water for several weeks or months, it is possible that if infected lobsters were translocated, microsporidians could become established in the environment, and/or in wild populations of crustaceans or invertebrate vectors such as polychaetes. While some microsporidians infecting crustaceans may exhibit high host specificity, others such as *Hepatospora* spp. may have a broad host range within the decapods (Bateman et al. 2016). If an index case occurred, the disease agent would likely persist in the population as the susceptible species would be unlikely to suffer epizootic mortalities, allowing establishment of translocated microsporidians into new geographic regions (Jones and Lawrence 2001, Stentiford et al. 2011). Given that microsporidians could occur in lobster broodstock and these disease agents can be horizontally transmitted within hatchery environments in the absence of appropriate biosecurity precautions, and environmental conditions are likely to be suitable for disease transmission in the wild in northern Australia, the risk of exposure and establishment is non-negligible, and the overall likelihood of exposure and establishment of microsporidians is considered to be **Moderate**.

5.4.7 Consequence assessment

In regions where microsporidians have infected farmed penaeid prawns, they have caused significant economic losses due to growth retardation and poor food conversion ratios (Santhoshkumar et al. 2016, Tang et al. 2016). In contrast, there is little known about the course of microsporidian disease in TRL, however it is thought that infection is likely to be terminal and progressive with eventual host death occurring due to overwhelming numbers of parasites interfering with normal organ functions. In any case, affected lobsters become unmarketable due to the damage inflicted by microsporidian infection of the valuable tail muscle. The significance of microsporidian infections as drivers of mortality in wild crustaceans has not been assessed (Palenzuela et al. 2014), however it is known that some microsporidians (e.g. *Hepatospora*) have low host specificity and may be able to infect a wide variety of decapod crustaceans (Bateman et al. 2016). It is also suspected that populations of wild crustaceans adversely affected by environmental stressors (e.g. adverse environmental conditions, or exposure to pollutants such as pesticides and herbicides) may experience reduced resilience due to microsporidian infection. As effects of disease in wild populations vary greatly due to factors such as environmental characteristics, host susceptibility and host densities (Burge et al. 2016), any adverse effects could result in ecological harm to aquatic environments, potentially resulting in cultural and socio-economic harm to

regional communities in northern Australia. There is no evidence to date that suggests that microsporidians that infect crustaceans present any risk to human health, however humans are susceptible to infection by Microsporidia, and the potential for host switching between invertebrates and humans may not be negligible (Snowden 2004, Stentiford et al. 2016).

As microsporidosis is a reportable disease in some jurisdictions (WA, ACT), their emergence in lobster culture systems may have implications for domestic trade. Indeed, a microsporidian disease outbreak in lobsters in northern Australia may require intervention by government authorities and disruption to normal trade in crustacean commodities by commercial fisheries and crustacean gathering by recreational fishers if attempts were made to limit potential spread into uninfected areas. However, once a microsporidian disease outbreak was detected, unless it was in an enclosed system there would appear to be minimal chance of eradication. Taking all of these factors into consideration, the environmental and human health impacts of emergence of microsporidian diseases in TRL are not entirely clear, while impacts on domestic industries and trade may be significant. The overall consequences of introduction and establishment of a microsporidian disease agent into the environment of northern Australia via the identified risk pathways are therefore likely to be **Moderate**.

5.4.8 Risk estimation

The unrestricted risk associated with microsporidosis is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The unrestricted risk estimate for microsporidosis exceeds the ALOP for at least one of the pathways examined, suggesting that additional risk management is required for these disease agents.

Risk estimate for Microsporidosis

Pathways	Via broodstock into hatchery in north QLD	Via water into hatchery in north QLD	Via juveniles into waters of QLD	Via juveniles into waters of the NT	Via juveniles into waters of WA
Combined likelihood of release and exposure	Very low	Low	Low	Low	Low
Consequences of establishment	Moderate	Moderate	Moderate	Moderate	Moderate
Risk estimation	Very low risk 6	Low risk 9	Low risk 9	Low risk 9	Low risk 9

5.5 Haplosporidosis

5.5.1 Aetiologic agent: Parasites of the genus *Haplosporidium* are protozoans which are members of the Order Haplosporida in the Phylum Endomyxa (Arzul and Carnegie 2015, Adl et al. 2019). Species of *Haplosporidium* infect mainly connective tissues and epithelia of invertebrates including molluscs, tunicates, annelid worms and crustaceans (Burreson and Ford 2004, Urrutia et al. 2019, Davies et al. 2020b). Most *Haplosporidium* species develop spores with an apically hinged operculum and other ornaments (Azevedo and Hine 2016). Haplosporidians (besides *Bonamia* spp.) have unknown, probably indirect life cycles, possibly requiring alternate (possibly planktonic) hosts (Haskin and Andrews 1988, Powell et al. 1999, Hartikainen et al. 2014).

5.5.2 Under official control in Australia: SA, WA, NT **Zoonotic:** No

5.5.3 Australias status: Haplosporidosis has been recorded in several taxa, including pearl oysters (*Pinctada maxima*) and tropical rock oysters (*Saccostrea cucullata*) in WA infected with *Haplosporidium hinei* (see Hine and Thorne 1998, Bearham et al. 2008a, 2008b) and *Minchinia occulta* (see Bearham et al. 2007, 2008a, 2008c), respectively. Sydney rock oysters (*S. glomerata*) from the Georges River, Port Stephens and Pambula River were also positive for a *Haplosporidium* sp. at high prevalences (31.8- 87.5%) using cPCR (Carnegie et al. 2014), while in crustaceans *Haplosporidium acetes* was found infecting the hepatopancreas of jelly prawns *Acetes sibogae australis* from Moreton Bay, Australia (Diggles et al. submitted).

5.5.4 Epizootiology

The Order Haplosporidia is composed of histozoic and coelozoic parasites that infect a wide variety of freshwater and marine invertebrates worldwide. There are currently four recognised haplosporidian genera (*Bonamia*, *Minchinia*, *Urosporidium* and *Haplosporidium*) (see Burreson and Ford 2004, Hartikainen et al. 2014, Azevedo and Hine 2016). There are at least 34 described species of *Haplosporidium*, but molecular analyses suggest the genus is paraphyletic (Burreson and Ford 2004, Arzul and Carnegie 2015, Azevedo and Hine 2016, Catanese et al. 2018) and has at least 3 clades (Urrutia et al. 2019). To date haplosporidian infections are best known from molluscs, in which infection by *Haplosporidium* spp. parasites has resulted in economically and ecologically significant mass mortalities in many parts of the world (Burreson and Ford 2004). For example, in the USA *Haplosporidium nelsoni* causes MSX disease which since 1957 has resulted in massive epizootics of eastern oysters (*Crassostrea virginica*) in high salinity (> 15‰) areas along the east coast of the United States (Andrews 1968, 1982, Haskin and Ford 1982, Burreson et al. 2000). In *Crassostrea gigas* in China (Wang et al. 2010) and *C. virginica* growing in water > 25‰ on the east coast of the USA, *H. nelsoni* sometimes occurs in mixed infections with the closely related *Haplosporidium costale*, which has also been detected in *C. gigas* on the US west coast following translocation of oysters (Burreson and Stokes 2006). Similarly, *H. nelsoni* was probably translocated to the east coast of the USA from Japan through imports of live *Crassostrea gigas* spat (Friedman 1996, Burreson et al. 2000, Kamaishi and Yoshinaga 2002). Reports of *H. nelsoni* from *Crassostrea gigas* in France (Renault et al. 2000) provide further evidence this parasite has been moved with translocation of infected oysters, despite the fact *H. nelsoni* has an unknown, indirect lifecycle that probably requires at least one intermediate host(s) for transmission (Haskin and Andrews 1988, Barber and Ford 1992, Ford et al. 2001, 2018). Modelling suggests that the infective stage of *H.*

nelsoni is water borne and most likely acquired by feeding (Haskin and Andrews 1988). Environmental studies have found *H. nelsoni* DNA in up to 70% of tunicates (*Styela* sp.) and about 30% of plankton samples in areas near where diseased *C. virginica* are cultured, however their role in the disease process as true hosts or mechanical vectors remains unclear (Messerman and Bowden 2016). Nevertheless, it has been pointed out that movements of putative alternative or reservoir hosts by ballast water or shipping could also have been the mechanism of spread of *H. nelsoni* to new locations (Burreson et al. 2000, Ford et al. 2018).

Besides Bonamiosis caused by *Bonamia* spp. (which for the sake of brevity will not be discussed here), other haplosporidian infections from molluscs include an undescribed New Zealand abalone parasite (NZAP) which caused mortalities up to 90% in an abalone (*Haliotis iris*) culture facility in New Zealand (Diggles et al. 2002b, Hine et al. 2002). This parasite contained rickettsiales-like prokaryotes in its cytoplasm (Hine et al. 2002) and molecular and ultrastructural analysis suggest that it falls at the base of the Phylum Haplosporidia (Reece et al. 2004, Hine et al. 2009, Arzul and Carnegie 2015, Azevedo and Hine 2016, Hine 2020). The inability to transmit infection horizontally or directly through inoculation (Diggles et al. 2002b) suggested that, like many other haplosporidians (Haskin and Andrews 1988, Powell et al. 1999, Bower and Meyer 2002, Burreson and Ford 2004), an intermediate host is probably required for completion of the lifecycle of the NZAP. In Australia, haplosporidians of the genus *Haplosporidium* and *Minchinia* have caused sporadic but heavy mortalities in hatchery reared pearl oysters (*Pinctada maxima*) and wild tropical rock oysters (*Saccostrea cucullata*) in Western Australia (Hine and Thorne 1998, 2000, 2002, Jones and Creeper 2006). *Haplosporidium hinei* was first found in 6 out of 106 pearl oysters (*Pinctada maxima*) spat 5-10 mm in shell height from a hatchery at Oyster Creek, Canarvon in northern WA in the early 1990s (Hine and Thorne 1998). By the time the presence of the infection was detected, however, the remaining spat had been moved to a grow-out area, where they apparently all died (Hine and Thorne 1998). A second detection in December 1995 found the same parasite at a prevalence of 4.6% in pearl oyster spat deployed to a nursery area north of Broome (Jones and Creeper 2006, Bearham et al. 2008b). By the time the oysters were destroyed 15 days later, the prevalence had increased to 10% (Jones and Creeper 2006). *Haplosporidium hinei* is thus considered to be a serious threat to the pearl industry (Bearham et al. 2008b, 2009a, 2009b). The second parasite originally observed by Hine and Thorne (2000), in samples of diseased *S. cucullata* from northern WA in 1993-94 has been associated with mortalities of up to 80% in wild rock oysters around Exmouth Island (Hine and Thorne 2000, Bearham et al. 2007) and was eventually described as *Minchinia occulta* (see Bearham et al. 2007, 2008a, 2008c). Mixed infections of *M. occulta* and *H. hinei* have also been recorded during disease outbreaks in hatchery reared *P. maxima* (see Bearham et al. 2009a).

Haplosporidians found in molluscs overseas have also caused disease in limpets (Di Giorgio et al. 2014, Ituarte et al. 2014), mussels (Molloy et al. 2012, Ward et al. 2019) and fan mussels (*Pinna noblis*) (see Catanese et al. 2018). Most relevant to the situation with TRL in northern Australia, however, is the fact that haplosporidians are also known to cause disease in various species of crustaceans. Some examples include *Haplosporidium louisiana* and *Haplosporidium cadomensis* which were found to infect the haemolymph and connective tissues of crabs (*Panopeus herbstii*, *Rhithropanopeus harrisii*) from north America and France, respectively (see Sprague 1963, Perkins 1975, Marchand and Sprague 1979). A *Minchinia*-like haplosporidian was found in the opaque haemolymph of two moribund blue crabs (*Callinectes sapidus*) from the east coast of North America (Newman et al. 1976). A "spot prawn parasite" was also found infecting the haemolymph of the Alaskan spot shrimp (*Pandalus platyceros*) and

pink shrimp (*Pandalus borealis*) (see Meyers et al. 1994, Reece et al. 2000, Bower and Meyer 2002), while other haplosporidians have been described infecting the hepatopancreas of white shrimp (*Penaeus vannamei*) (see Dykova et al. 1988, Nunan et al. 2007, Utari et al. 2012), causing systemic disease in the European shore crab (*Carcinus maenas*) due to infections by *Haplosporidium littoralis* (see Stentiford et al. 2004, 2013). Other haplosporidians described from *C. maenas* include *H. carcini* and *H. cranc* (see Davies et al. 2020b), while novel haplosporidians infecting the connective tissues have caused motor impairment and reduced fitness in amphipods (Larsson 1987, Winters and Faisal 2014, Urrutia et al. 2019).

The first haplosporidian reported from Australian crustaceans was found in 2018 in wild jelly prawns (*Acetes sibogae australis*) from Dux Creek in northern Moreton Bay, QLD, which displayed grossly visible opacity of the hepatopancreas. Affected jelly prawns were sampled and examined histologically, revealing massive infection by multinucleate plasmodia of a haplosporidian-like parasite in the epithelial cells of the hepatopancreas (Diggles 2020a, Diggles et al. submitted). The parasite was identified as a new species of haplosporidian, the first report of haplosporidiosis in sergestid shrimp, and the parasite was named *Haplosporidium acetes* (see Diggles et al. submitted). Infections of *H. acetes* were observed in all cell types (R, B, F and E) within the hepatopancreas, with infected epithelial cells becoming hypertrophied as they filled with haplosporidian plasmodia, causing almost complete displacement of normal hepatopancreas tissue in heavy infections (Diggles et al. submitted). Although sporulation was not observed, infected jelly prawns appeared terminally diseased. Infections became grossly evident in around 5% of wild prawns during early autumn at a time of year when jelly prawn populations decline rapidly with decreasing water temperatures, however histopathology indicated at least 13% of apparently normal jelly prawns were also infected, suggesting that population prevalence approached or exceeded 20% (Diggles 2020a, Diggles et al. submitted).

Infections by haplosporidians are usually systemic and terminal (Hine and Thorne 1998, 2002, Diggles et al. 2002b), and haplosporidian infections of crustaceans are no exception. For example, Utari et al. (2012) reported that white shrimp (*P. vannamei*) cultured in Indonesia became heavily infected with a haplosporidian parasite closely related to *H. acetes* which infected the epithelium of the hepatopancreas. Infected *P. vannamei* experienced high mortality and slow growth, resulting in overall survival rates in some affected shrimp ponds as low as 10% (Utari et al. 2012). Prevalence of infections in broodstock and ponds from 2004 to 2010 suggested that the haplosporidian disease outbreaks had resulted from stocking of infected post-larvae. *Haplosporidium littoralis* causes severe alterations to the connective tissues and haemolymph of infected European shore crabs (Stentiford et al. 2004, 2013), and the infection was considered likely to be terminal and a mortality driver in populations of *Carcinus maenas* (see Stentiford et al. 2013). In contrast, *H. carcini* and *H. cranc* do not seem to cause disease in the same host (see Davies et al. 2020b). In Moreton Bay, *Haplosporidium acetes* infections became grossly evident in jelly prawns during early autumn, at a time of year when jelly prawn populations normally decline rapidly at that location due to decreasing water temperatures (Diggles et al. submitted). Given the fact that *H. acetes* caused severe damage to the hepatopancreas (in a similar manner to the haplosporidian described by Utari et al. (2012) which caused substantial mortality in cultured *P. vannamei*), it is likely that *H. acetes* can also cause significant disease and mortality, such that it could significantly influence jelly prawn population dynamics (Diggles et al. submitted).

Haplosporidian vegetative stages can proliferate within host connective tissues forming masses of multi-nucleated plasmodia, while sporulation usually occurs within the epithelia of the digestive tract (Bearham et al. 2008b). Eventual host death is thought to be due to overwhelming numbers of parasites interfering with normal organ functions. It appears unlikely that haplosporidian vegetative cells can survive freezing, however the freeze tolerance of spore stages remains unknown (Diggles 2011). Nevertheless, the spores of haplosporidians are thick walled and are considered highly likely to be robust and environmentally persistent (Ford et al. 2018). The inability to transmit haplosporidians directly by cohabitation or injection of spores means little is known about the longevity or robustness of their infective stages, and it suggests they have an indirect lifecycle requiring one or more alternate hosts (Diggles et al. 2002b, Bower and Meyer 2002, Burreson and Ford 2004, Ford et al. 2018) which may be planktonic (Hartikainen et al. 2014). In bivalves the earliest vegetative stages of *H. nelsoni* are found in the epithelia of the gills and palps, suggesting that the infective stage is waterborne (Haskin and Andrews 1988). Neither the infective stage nor the mode of transmission of *H. nelsoni* has ever been identified (Powell et al. 1999, Sunila et al. 2000, Ford et al. 2018), although it is known that the infective stage for *H. nelsoni* can pass through a 1 mm filter (Sunila et al. 2000), and a 150 µm filter, but not a 1 µm filter followed by UV irradiation at a dose of 30 mJ/cm^2 (Ford et al. 2001).

5.5.5 Release assessment

Filter feeding bivalves are efficient particle collectors which can concentrate haplosporidian life stages which may occur in the environment (Ford et al. 2009). Indeed, Ford et al. (2018) reported how *H. nelsoni* DNA is regularly found in a high percentage of water and sediment samples, as well as a wide range of invertebrates (but not crustaceans) using cPCR, but these did not appear to be true infections but instead probably represented various invertebrates acting as mechanical vectors. Carnegie et al. (2014) found a *Haplosporidium* sp. in *S. glomerata* from the Georges River, Port Stephens and Pambula River in NSW at high prevalences (31.8- 87.5%) using cPCR. However, they found no histological evidence of infection by recognizable *Haplosporidium* sp. life stages in any of the material they examined. The emergence of new haplosporidian diseases in Australian oyster species such as *M. occulta* and *H. hinei* in *S. cuccullata* and *P. maxima*, together with the recent detection of *H. acetes* in jelly prawns in south east QLD (Diggles et al. submitted) together demonstrate that crustaceans in Australia are being exposed to haplosporidians that naturally occur in the coastal environment. However, very little is known about the full range of haplosporidian infections present in the various species of crustaceans in Australia at this time. In northern Moreton Bay, *H. acetes* has been recorded in the same population of jelly prawns in Dux Creek for over 4 years running (2018-2021) at prevalences that may exceed 20% (Diggles et al. submitted), but opaque hepatopancreases were not observed in jelly prawns sampled from other parts of Moreton Bay during surveillance for WSSV vectors (Diggles 2020c), suggesting that the distribution of this parasite may be patchy on small spatial scales. *Haplosporidium* spp. are also known to be present in the waters of northern WA, but infections by *H. hinei* in *P. maxima* in WA have only been recorded a handful of times, at relatively low prevalence (<10%) (Hine and Thorne 1998, Jones and Creeper 2006, Bearham et al. 2008b, 2009a, 2009b). Because of this, the risk that *Haplosporidium* spp. may occur in TRL in northern Australia remains non-negligible.

Transmission of *H. nelsoni* into hatcheries occurs via intake water (Sunila et al. 2000) but is preventable by particle filtration down to 1 µm or less followed by UV irradiation to a minimum of 30 mJ/cm^2 (Ford et al. 2001), suggesting that normal water treatment in hatcheries should be sufficient to exclude

Haplosporidium spp. that may occur in intake water. This is important, given that *Haplosporidium* spp. can proliferate rapidly when hosts are held at high densities in captivity (Hine and Thorne 1998, Sunila et al. 2000). This is despite the fact that there is little evidence that *Haplosporidium* spp. can be transmitted horizontally through the water or vertically between generations. Indeed, given that the lifecycle of these parasites is likely to be indirect (Ford et al. 2018), true vertical transmission is not likely to occur within the hatchery environment, however pseudo-vertical transmission may be still possible via crustaceans contacting waterborne vegetative stages, so it may still be possible that these parasites could be transmitted in hatcheries if larvae or juvenile crustaceans were spawned from infected broodstock (Utari et al. 2012) or in water where haplosporidian vegetative stages have not been filtered out or inactivated. The vegetative stages of haplosporidians may also be more resistant to UV exposure than the infective stages, as shown by studies of Fernandez-Boo et al. (2021) who found a minimum UV dose of 94 mJ/cm^2 was required to inactivate cells of the haplosporidian *Bonamia ostreae*. Taking into account the information above, the risk of release is not negligible, and unrestricted likelihood estimations for the release of *Haplosporidium* spp. into northern Australian waters via the various release pathways are provided below.

Release assessment for Haplosporidosis

Pathways	Via broodstock into hatchery in north QLD	Via water into hatchery in north QLD	Via juveniles into waters of QLD	Via juveniles into waters of the NT	Via juveniles into waters of WA
Likelihood of release	Very low	Moderate	Low	Low	Low

5.5.6 Exposure assessment

Wild and cultured crustaceans in northern Australia are already at risk of exposure to haplosporidians, however, introduction of *Haplosporidium* sp. via the proposed translocations could increase their risk of exposure, but only if sufficient viable infective particles were introduced into areas where susceptible intermediate hosts and/or crustacean final hosts were present under environmental conditions suitable for transmission. At least one haplosporidian in Australia (*H. acetes*) is known to be able to infect crustaceans, however in the absence of knowledge of the identity of its lifecycle and likely existence of intermediate host(s) it is not possible at this time to determine whether wild crustaceans or other invertebrates besides jelly prawns may be mechanical vectors, carriers or susceptible to disease caused by *H. acetes*.

Because an intermediate host is presumably needed in order to complete the lifecycle of haplosporidians, the exposure pathway required for transmission remains unknown, as does important information such as the minimum infective dose required for an index case to occur, though being a parasite theoretically infection by just one infective stage can result in successful transmission. However, if an index case occurred, these disease agents are highly pathogenic and it would be likely that the infected crustacean would become diseased, after which transmission and further spread from the index case would be possible. The current restricted distribution of some of the known haplosporidian pathogens of oysters and crustaceans may be due to the fact that their intermediate hosts may also be restricted in distribution. However, the fact that other species of Haplosporidians (e.g. *Haplosporidium nelsoni*) have been translocated and established infections in new regions (Friedman 1996, Burreson et al. 2000, Renault et al. 2000), suggests that some of the presumptive intermediate hosts may be widespread and/or ubiquitous

(e.g. planktonic copepods, Hartikainen et al. 2014), or that these parasites may have lower host specificity for the intermediate host. Taking these various factors into consideration, the risk of exposure and establishment of haplosporidians via the proposed translocations remains non-negligible, and the overall likelihood of exposure and establishment of *Haplosporidium* spp. is considered to be **Low**.

5.5.7 Consequence assessment

Although haplosporidian parasites are already present in populations of wild crustaceans in some areas of northern Australia, the distribution of these parasites may be patchy and other regions may be free of infection at this time. There is evidence that haplosporidians can cause major disease outbreaks and significant impacts on populations of cultured crustaceans (Nunan et al. 2007, Utari et al. 2012), and a high proportion of wild crustaceans infected with haplosporidians (often at prevalences that approach or exceed 20%) appear to be terminally diseased (Meyers et al. 1994, Bower and Meyers 2002, Stentiford et al. 2004, 2013). Within Australia, in the case of *H. acetes* in wild jelly prawns, the presence of the parasite coincides with seasonal population declines in its host, suggesting that it may even play a role in regulating the host population (Diggles et al., submitted). Haplosporidosis is no longer listed by the OIE and NACA, but these disease agents remain listed as a reportable disease in SA, WA and the NT (Table 2). Hence the spread of haplosporidian parasites to new areas could adversely impact trade as well as pose a significant obstacle to future investment in crustacean aquaculture in northern Australia. Considering all of these factors, establishment of *Haplosporidium* spp. in new areas would likely have significant biological consequences for crustacean aquaculture and could cause economic harm together with significant environmental effects through mortality of wild crustaceans. Furthermore, once these disease agents are detected in the wild, there would appear to be little chance of eradication. If the experience following the introduction of *H. nelsoni* into the east coast of the USA is any guide, development of meaningful resistance to *Haplosporidium* spp. infections following their introduction into wild host populations may take 50 years or more (Carnegie and Burreson 2011, Ford and Bushek 2012). It is therefore considered that the consequences of introduction of *Haplosporidium* spp. into the waters of northern Australia via the identified risk pathways would likely be **High**.

5.5.8 Risk estimation

The unrestricted risk associated with haplosporidosis is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The unrestricted risk estimate for haplosporidosis exceeds the ALOP for all pathways, suggesting that additional risk management is required for these disease agents.

Risk estimate for infection with Haplosporidosis

Pathways	Via broodstock into hatchery in north QLD	Via water into hatchery in north QLD	Via juveniles into waters of QLD	Via juveniles into waters of the NT	Via juveniles into waters of WA
Combined likelihood of release and exposure	Very low	Low	Very low	Very low	Very low
Consequences of establishment	High	High	High	High	High
Risk estimation	Low risk 8	Moderate risk 12	Low risk 8	Low risk 8	Low risk 8

5.6 Infection with *Hematodinium* spp.

5.6.1 Aetiologic agent: *Hematodinium australis* and other parasitic dinoflagellates of the genus *Hematodinium* (Order Syndinida, Family *Syndiniceae*).

5.6.2 Under official control in Australia: No **Zoonotic:** No

5.6.3 Australias status: *Hematodinium australis* has been reported from a range of crab species in QLD (Shields 1992, Hudson and Lester 1994, Hudson and Shields 1994, Hudson and Adlard 1994, 1996), while *Hematodinium*-like agents have also been reported from blue swimmer crabs (*Portunus armatus*) in Shark Bay in Western Australia (Diggles 2011, Small 2012), in 3 crab species in Victoria (Gornik et al. 2013) and in *P. armatus* in Gulf St Vincent in SA (Beckmann and Hooper 2015).

5.6.4 Epizootiology

Dinoflagellates in the genus *Hematodinium* are economically important algal parasites which cause fatal disease in a broad range of wild marine decapod crustaceans, particularly crabs and lobsters (Stentiford and Shields 2005, Small 2012, Shields 2019). More recently, however, they have begun to be problematic in the culture of various species of captive crustaceans in Asia, including mud crabs (*Scylla serrata*, *Scylla paramamosain*), gazami (coral) crabs (*Portunus trituberculatus*), mudflat crabs (*Helice tientsinensis*), and both penaeid and palaemonid prawns (Li et al. 2008b, 2013, 2021, Xu et al. 2010, Wang et al. 2017, Huang et al. 2019). The genus *Hematodinium* was first described by Chatton and Poisson (1931), who found *Hematodinium perezi* in the haemolymph of diseased *Carcinus maenas* and *Liocarcinus depurator* in France. Several studies have since described major *Hematodinium* spp. epizootics that have damaged fisheries for a wide range of species of decapod crustaceans in many countries (Messick 1994, Stentiford and Shields 2005, Shields 2012, 2019, Small 2012, Li et al. 2021), particularly temperate fisheries for American blue crabs (*Callinectes sapidus*) along the east coast of the USA (Newman and Johnson 1975, Messick 1994, Messick and Shields 2000, Small et al. 2019a), the Norway lobster *Nephrops norvegicus* in the North Sea and north Atlantic (Field et al. 1992, Field and Appleton 1995, Stentiford and Neil 2011), velvet swimming crabs (*Necora puber*), edible crabs (*Cancer pagurus*) and harbour crab (*Liocarcinus depurator*) in Europe (Wilhelm and Mialhe 1996, Stentiford et al. 2002, Small et al. 2012), king crabs *(Paralithodes camtschaticus* and *P. platypus*), tanner crab *Chionoecetes bairdi*, and spiny king crab *Paralithodes brevipes* in the Sea of Okhotsk and Bering Seas in Russia (Small 2012, Ryazanova et al. 2021), as well as tanner crabs and snow crabs (*Chionoecetes opilio*) in Alaska, British Columbia and off Newfoundland (Meyers et al. 1987, Small 2012).

Crabs and lobsters infected by *Hematodinium* sp. undergo dramatic pathological alterations to their organs, tissues and haemolymph and eventually die (Meyers et al. 1987, Field et al. 1992, Stentiford and Shields 2005). *Hematodinium* infections in the Norway lobster (*Nephrops norvegicus*) are associated with moribund lobsters displaying an abnormal dull orange colouration, with 'watery' muscles, low haemolymph pressure and milky-white body fluids (Field et al. 1992). Other species exhibit similar signs of discolouration of the carapace, milky white body fluids and haemolymph that does not clot, and a “chalky” or “cooked” appearance of the flesh, with the external signs of infection accompanied by several physiological and biochemical disruptions to the muscles and other organs which substantially alter the metabolism of infected hosts (Stentiford and Shields 2005, Stentiford et al. 2015). The condition caused by infection with *Hematodinium* spp. is known around the world by its various gross signs in different

hosts, including such terms as “bitter crab disease”, “pink crab disease”, “milky disease”, "milky blood disease", "milky shrimp disease" and “yellow water disease” (Small 2012).

In Australia, several crab species are known to be naturally infected with *Hematodinium australis*, including *Portunus pelagicus/armatus*, and *Scylla serrata* in Moreton Bay and *Trapezia areolata* and T. *coeruleab* from the Great Barrier Reef (see Shields 1992, Hudson and Shields 1994, Hudson and Adlard 1994, 1996, Small 2012). Gornik et al. (2013) used a genus specific DNA probe to detect *Hematodinium* sp. in sand crabs (*Ovalipes australiensis*), giant spider crabs (*Leptomithrax gaimardii*) and red bait crab (*Plagusia chabrus*) from Port Phillip Bay in Victoria. *Hematodinium*-like agents have also been reported from blue swimmer crabs (*Portunus armatus*) in Gulf St Vincent in SA (Beckmann and Hooper 2015) and Shark Bay in Western Australia (Diggles 2011, Small 2012, Li et al. 2021). Hudson and Shields (1994) differentiated *Hematodinium australis* from the type species *H. perezi* on the basis of size of the vegetative stage (trophont), the presence of rounded plasmodial stages and the southern hemisphere location of *H. australis*. Molecular studies by Hudson and Adlard (1996) later supported the separation of *H. australis* from *Hematodinium perezi*. The *Hematodinium* sp. found infecting sand crabs, giant spider crabs and red bait crabs in Port Phillip Bay at high prevalences (53-87%) using PCR may also be *H. australis*, but this needs further verification as the gene probe used in that study was only genus specific (Small et al. 2007, Gornik et al. 2013). On the other hand, Small (2012) suggested that the taxonomy of *H. australis* needs to be revisited with modern molecular tools, given that there are at least 3 intraspecific genotypes (clades) of *H. perezi* in different host groups in different geographic areas (Jensen et al. 2010, Li et al. 2021), meaning that the species concept within the genus *Hematodinium* remains to be fully defined.

Virtually all of the Syndinida are parasitic in the haemocoels of invertebrate hosts. The *Hematodinium* lifecycle consists of at least 3 phases (Stentiford and Shields 2005): a multinucleate plasmodial stage, a vegetative phase (trophont, produced via merogony) and an asexual reproductive phase (sporont produced via sporogony). In the Syndinida, sporogony leads to the formation of 2 dissimilar forms of biflagellate dinospores (‘swarmers’) infective stages that arise from different parent infections and ensure dispersal and new infection. *In-vitro* cell culture of *Hematodinium* found numerous vegetative life-history stages including filamentous trophonts, amoeboid trophonts, arachnoid trophonts, arachnoid sporonts, sporoblasts, prespores and motile biflagellated dinospores including macro-dinospores (11-17 µm) and micro-dinospores (6-12 µm) (Li et al. 2011b). Within the decapod host *Hematodinium* cells occur primarily as plasmodial forms that divide and grow until they undergo sporogony to produce a motile spore stage. The plasmodial stage has no chloroplasts and obtains nutrition via osmotrophy during the trophic phase, where lipid and polysaccharide inclusions suggest active feeding at the expense of the host (Stentiford and Shields 2005). Sporogenesis is simple with multiplication of the nuclei, plasmodial and cytoplasmic divisions occurring to produce sporocysts, from which the infective biflagellate dinospores are produced and liberated (Li et al. 2011b). Mortality rate of infected crabs is often 100% (Meyers et al. 1996) and sporulation is usually followed by death of the host (Stentiford and Shields 2005). Mortality of juvenile blue crabs (*C. sapidus*) infected with *H. perezi* was found to be 10 times higher at elevated temperatures (25 and 30 °C) and salinity (30 ‰) compared to uninfected crabs, indicating that early benthic juveniles will experience significant mortality due to *H. perezi* with increasing ocean temperatures (Huchin-Mian et al. 2018, Shields 2019).

No resting cyst stages of the life cycle have been reported to date, though their presence cannot be ruled out (Stentiford and Shields 2005). The parasite nevertheless appears to persist in host populations year

round, usually with seasonal peaks at high prevalences. For example, in *Callinectes sapidus* off Florida, USA, clinical *Hematodinium* sp. infections reached a peak prevalence of 30 % (Newman and Johnson 1975). Messick (1994) subsequently reported an epizootic of *Hematodinium* that affected 70 to 100% of the juvenile *Callinectes sapidus* in the seaside bays of Maryland and Virginia in 1991 and 1992. Later studies found that prevalence of *Hematodinium* sp. infections followed a seasonal pattern, with a sharp peak in late autumn with highest prevalence in wild crabs less than 30 mm carapace width (Messick and Sheilds 2000, Frischer et al. 2006). Prevalences did not vary with moult stage, but were highest in crabs collected from salinities of 26 to 30‰ with no infections below 11‰ salinity (Messick and Shields 2000). In France, *Hematodinium perezi* infections were associated with winter crab mortalities, with peak prevalences of clinical disease in velvet crab *Necora puber* observed to be as high as 87%, resulting in a catastrophic 96% decline in the local fishery (Wilhelm and Boulo 1988, Wilhelm and Mialhe 1996). Infections of commercially fished populations of tanner and snow crabs in the Bering Sea and southeast Alaskan waters with *Hematodinium* sp. were reported with peak prevalences approaching 100 % (Meyers et al. 1990, 1996, Eaton et al. 1991). Evidence from the snow crab (*C. opilio*) fishery in Newfoundland, Canada, showed the prevalence of *Hematodinium* sp. had increased steadily from 0.037% to 4.25% over a 10 year period (Pestal et al. 2003), affecting over 9% of males and 25% of females in an epizootic occurring in Conception Bay in 2000 (Shields et al. 2005). Infections by *Hematodinium* sp. in host populations are usually seasonal and peak during the late summer or autumn months. For example, *Hematodinium* sp. was detected in cultured *P. trituberculatus* and wild mudflat crabs *Helice tientsinensis* near polyculture ponds in China at prevalences between 16-72.9% and 5.8–31.7%, respectively, with peak prevalence during the summer wet season months when water temperatures were high and rains caused dramatic environmental changes (Huang et al. 2019, Li et al. 2021). Similarly, the prevalence of *Hematodinium* sp. in wild *Nephrops norvegicus* in Scotland also peaked around 70% in springtime (Field et al. 1992).

It is notable that outbreaks of disease due to *Hematodinium* sp. in wild populations of crustaceans tend to occur in areas with entrained water masses such as lagoons, embayments or fjords with shallow sills (Meyers et al. 1987, 1990, Eaton et al. 1991, Field et al. 1992, Messick 1994, Wilhelm and Miahle 1996). This has also been the case in Australia, where *Hematodinium* sp. has been found in crabs in Moreton Bay, Port Phillip Bay, Shark Bay and Gulf St Vincent. Clearly, given suitable inshore hydrographic conditions in bays with poor water circulation, *Hematodinium* sp. represents a significant threat to wild (and cultured) populations of decapod crustaceans. Nevertheless, the development of sensitive molecular diagnostic tools has found that in some circumstances, *Hematodinium* spp. can persist in populations of wild crustaceans at low prevalences in subclinical infections (Ryzanova et al. 2021).

Studies conducted in large bays along the east coast of the USA have found that larval stages of American blue crabs (*C. sapidus*) are uninfected, but they quickly become infected with *Hematodinium* soon after megalopae settle onto benthic substrates in high-salinity bays during the late summer and autumn months (Small et al. 2019a). Naïve juvenile crabs introduced into bays where *Hematodinium* sp. is endemic in wild decapods quickly become infected within the first 3-10 days (Huchin-Mian et al. 2017). Infected juvenile crabs then overwinter with the parasite and, when subjected to increasing water temperatures in spring, infections progress rapidly, culminating in transmission to other crabs in late spring and early summer (Small et al. 2019a). While the vast majority of the disease outbreaks caused by *H. perezi* have been recorded in wild decapods in high-salinity embayments (>18‰ salinity, Messick and Shields 2000), the discovery by Li et al. (2008b) of *Hematodinium* sp. causing disease in mud crabs (*Scylla paramamosain*, previously known as *Scylla serrata*), cultured in hyposaline conditions (< 9‰) in Asia

indicates that some forms of the parasite may also be able to cause disease in estuaries as well as oceanic conditions. Transmission in the wild occurs horizontally through the water via exposure to infective zoospores in the water column, while infection can also occur by injection of infected haemolymph and cannibalism (Messick and Shields 2000, Walker et al. 2009), though the latter not in all situations (Li et al. 2011a). Ameboid trophonts of *Hematodinium* sp. from *C. sapidus* showed reduced viability after 24 hours in seawater, whereas dinospores from naturally sporulating crabs were found to be able to survive up to 7 days in seawater at 21-23°C (Li et al. 2011a). Besides decapod crustaceans, *Hematodinium* spp. have also been found in amphipods, which may act as alternate or reservoir hosts (see Hudson and Shields 1994, Shields 1994).

5.6.5 Release assessment

Several wild crustacean species that occur along the east coast of Australia are known to harbour infections of *Hematodinium australis*, including *Scylla serrata* and *Portunus pelagicus* in inshore areas, as well as *Trapezia areolata* and T. *coeruleab* from the Great Barrier Reef (see Shields 1992, Hudson and Shields 1994, Hudson and Adlard 1994, 1996, Small 2012). *Hematodinium*-like agents have also been reported from blue swimmer crabs (*Portunus armatus*) in Shark Bay in Western Australia (Diggles 2011, Small 2012, Li et al. 2021). Earlier investigations using light microscope cytology for diagnosis found the prevalences of *H. australis* in inshore crab populations in Moreton Bay were low, up to 4% in *P. armatus* and 1.5% of *S. serrata* (see Hudson and Shields 1994, Hudson and Lester 1994). Infected crabs detected by cytology did harbour very high intensity *H. australis* infections, for example 1 x 10^6 *Hematodinium* cells/ml in the haemolymph of *P. armatus* (see Hudson and Shields 1994). However, recent studies of 3 crab species from Port Phillip Bay in Victoria used more sensitive PCR molecular diagnostic techniques to find *Hematodinium* sp. at much higher prevalences (53-87%) in subclinical infections (Gornik et al. 2013). The results from Gornik et al. (2013) therefore suggest that the actual prevalence of *H. australis* infection in crabs and potentially other decapod hosts in Moreton Bay, the GBR and other locations in QLD may be higher than earlier studies suggest. Infection dynamics of *Hematodinium* sp. in host populations also generally show strong host size and seasonal effects on parasite prevalence and intensity (Messick and Sheilds 2000, Huang et al. 2019, Small et al. 2019a). Because of this, even though *H. australis* and *Hematodinium* -like agents are already known to occur in some areas of northern Australia, in the absence of intensive targeted surveillance throughout the entire year, the true status of *Hematodinium* sp. infections of decapod crustaceans, including TRL, throughout northern Australia remains to be determined. Nevertheless, it appears highly likely that hatcheries and holding facilities situated in inshore regions of northern Australia would be exposed to *Hematodinium* spp. vectored by wild populations of crustaceans via the intake water. Taking into account the information above, the risk of release is not negligible, and unrestricted likelihood estimations for the release of *Hematodinium* spp. into northern Australian waters via the various release pathways are provided below.

Release assessment for infection with *Hematodinium* spp.

Pathways	Via broodstock into hatchery in north QLD	Via water into hatchery in north QLD	Via juveniles into waters of QLD	Via juveniles into waters of the NT	Via juveniles into waters of WA
Likelihood of release	Low	High	Low	Low	Low

5.6.6 Exposure assessment

Wild and cultured crustaceans throughout some parts of northern Australia are already at risk of exposure to *H. australis* and *Hematodinium*-like agents that occur naturally in the Australian environment. However, translocation of large numbers of cultured juvenile TRL containing *Hematodinium* sp. could increase the risk of exposure of wild crustaceans in northern Australia and transport these disease agents into new regions. Nevertheless, infection and establishment in new hosts would occur only if viable *Hematodinium* sp. was introduced into an area where susceptible hosts were present under suitable environmental conditions for transmission. Inoculation experiments have shown that all stages of the *Hematodinium* lifecycle, including filamentous trophonts, vegetative amoeboid trophonts, macro-dinospores and micro-dinospores are capable of establishing new infections (Meyers et al. 1987, Eaton et al. 1991, Hudson and Shields 1994). Meyers et al. (1996) found potential evidence for sexual transmission of *Hematodinium* in *Chionoecetes bairdi* with parasites present in the seminal fluids of the vas deferens in a few males, however the apparent absence of *Hematodinium* sp. in the planktonic larval stages of *C. sapidus* suggests that vertical transmission of the disease is unlikely with crustaceans most likely becoming infected by contact with infective dinospores soon after benthic settlement and metamorphosis into post larvae or juveniles (Huchin-Mian et al. 2017, Small et al. 2019a). Infection by cohabitation through horizontal transmission of dinospores is possible and may be the most common route of natural infection (Stentiford and Shields 2005, Frischer et al. 2006). Some studies report infection via the *per-os* route through cannibalism (Walker et al. 2009), though this does not seem to occur in all hosts and situations (Hudson and Shields 1994, Li et al. 2011a). The minimum dose required for successful transmission of *Hematodinium* sp. is not well known, however the LD50 for *H. australis* infection in *P. armatus* and *S. serrata* (as shown by inoculation of healthy crabs with 0.05-0.1 ml of haemolymph containing 1.0 x 10^6 *Hematodinium* cells/ml), was around 0.5-1 x 10^5 *Hematodinium* cells, with all infected crabs dying within 16 days post-inoculation (Hudson and Shields 1994).

The infective stages of *Hematodinium* sp. remain viable in the environment for at least 1 week (Li et al. 2011a, extending their period of infectivity which would increase the risk of exposure. If susceptible species of wild lobsters or other susceptible hosts in northern Australia were exposed to viable infective stages of via one of the identified pathways, transmission and spread of these disease agents may occur as has been observed in populations of wild crustaceans, particularly those in entrained water masses such as lagoons, embayments or fjords with shallow sills flows (Stentiford and Shields 2005). This suggests that *Hematodinium* spp. is likely to become established after an index case occurs, although these events would likely be modulated to a certain extent by predation of moribund crustaceans by non susceptible species such as fish. Taking these various factors into consideration, given that *Hematodinium* sp. could occur in lobster broodstock and these disease agents can be horizontally transmitted within hatchery environments in the absence of appropriate biosecurity precautions, and environmental conditions are likely to be suitable for disease transmission in the wild in northern Australia, the risk of exposure and establishment is non-negligible, and the overall likelihood of exposure and establishment of *Hematodinium* sp. is considered to be **Moderate**.

5.6.7 Consequence assessment

Hematodinium sp. infections are highly pathogenic, with sub-clinical disease causing metabolic disturbances (Stentiford et al. 2015) generally progressing to clinical disease with most infected decapods dying within 4 to 40 days, depending on factors such as host size and water temperatures (Stentiford and

Shields 2005, Frischer et al. 2006, Walker et al. 2009, Huchin-Mian et al. 2018). At least one strain/species of *Hematodinium* is already known to be present in the Australian environment (Hudson and Shields 1994), but others may exist in different geographical locations (Gornik et al. 2013, Beckmann and Hooper 2015). Little is known about the distribution and epizootiology of *Hematodinium* sp. infections in Australian crustaceans, except for the fact to date there has been no evidence that these parasites have had any discernible impact on wild populations. However, in other regions of the world, *Hematodinium* infections have had significant detrimental impacts on fisheries and wild populations of crabs and lobsters (Wilhelm and Boulo 1988, Wilhelm and Mialhe 1996, Stentiford and Neil 2011, Shields 2012, Small 2012). More recently, *Hematodinium* spp. have become problematic in the aquaculture of various types of crustaceans in Asia, and hence their introduction into new areas may result in significant mortalities and ongoing financial losses to aquaculturists (Li et al. 2008b, 2013, 2021, Xu et al. 2010, Wang et al. 2017, Huang et al. 2019), especially as there are no methods of control available. Furthermore, as *Hematodinium* sp. infection causes more severe disease at higher water temperatures (Huchin-Mian et al. 2018), this parasite is likely to become even more problematic as the global warming trend continues (Shields 2019). *Hematodinium* spp. is not listed as a reportable disease by the OIE or NACA, and is also not listed as reportable in any State (Table 2), even though infection by *Hematodinium* spp. causes severe disease in both wild and cultured crustaceans, and as such meets the OIE criteria for listing as an emerging aquatic animal disease (Small 2012). Thus, at the present time, the spread of *Hematodinium* spp. to new areas is unlikely to adversely impact trade. Considering all of these factors, establishment of *Hematodinium* spp. into new areas would have significant consequences for aquaculture of susceptible crustaceans (particularly crabs and lobsters) potentially causing disease that would not be readily amenable to control, and its introduction into new regions could also cause significant biological consequences and environmental effects, as well as severe adverse economic consequences for crustacean fisheries. It is therefore estimated that the overall consequences of introduction and establishment of *Hematodinium* spp. into the environment of northern Australia via the identified risk pathways would likely be **Moderate**.

5.6.8 Risk estimation

The unrestricted risk associated with infection with *Hematodinium* spp. is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The unrestricted risk estimate for infection with *Hematodinium* spp. exceeds the ALOP for all pathways, suggesting that additional risk management is required for these disease agents.

Risk estimate for infection with *Hematodinium* spp.

Pathways	Via broodstock into hatchery in north QLD	Via water into hatchery in north QLD	Via juveniles into waters of QLD	Via juveniles into waters of the NT	Via juveniles into waters of WA
Combined likelihood of release and exposure	Low	Moderate	Low	Low	Low
Consequences of establishment	Moderate	Moderate	Moderate	Moderate	Moderate
Risk estimation	Low risk 9	Moderate risk 12	Low risk 9	Low risk 9	Low risk 9

5.7 Scuticociliate disease

5.7.1 Aetiologic agent: Marine scuticociliates (Protozoa: Ciliophora) that infect crustaceans, including members of the genera *Anophryoides* spp., *Lynnia* spp., *Mesanophrys* spp., and *Orchitophrya* spp. (see Morado and Small 1994, 1995, Morado et al. 1999, Small et al. 2005a, Miller et al. 2013, Liu et al. 2020, Metz and Hechinger 2021).

5.7.2 Under official control in Australia: No **Zoonotic:** No

5.7.3 Australias status: Pathogenic marine scuticociliate infections have been reported in finfish from QLD, SA and Tasmania (Munday et al. 1997, Diggles 2011), while aquatic scuticociliates have also caused disease in a wide range of freshwater fish species (Rowland and Ingram 1991, O'Donoghue and Adlard 2000, Herbert and Graham 2008), as well as red claw crayfish (*Cherax quadricarinatus*) in QLD (Edgerton et al. 1995, 1996).

5.7.4 Epizootiology

Systemic infections by scuticociliates are problematic in the culture and/or captive holding of a wide variety of marine taxa worldwide including not only crustaceans (Morado and Small 1994, 1995), but also starfish (Byrne et al. 1997), bivalve molluscs (Elston et al. 1999) and finfish (Munday et al. 1997, Smith et al. 2009). Scuticociliates are usually free living during which time they are microphagous, feeding mainly on bacteria, but under certain circumstances they can act as opportunistic pathogens which colonise external and internal organs of aquatic animals (Bower et al. 1994, Munday et al. 1997). Juvenile marine finfish and shellfish in aquaculture systems appear to be particularly susceptible to scuticociliate infection any time they are held at high densities in tanks that contain nutrient enriched water containing high numbers of bacteria (Bower et al. 1994, Liu et al. 2020). These conditions tend to increase scuticociliate densities to levels high enough to facilitate horizontal infection of fish or shellfish which may be injured or immunocompromised (Bower et al. 1994, Munday et al. 1997). Once scuticociliates gain a portal of entry into a host, they switch to a histophagous mode of nutrition, rapidly destroying infected tissues, resulting in disease and high rates of mortality (Munday et al. 1997, Miller et al. 2013, Liu et al. 2020). Control of these disease outbreaks in situations where hosts are held at high densities can be difficult, usually requiring reduction of nutrient loads in rearing tanks, removal of dead and dying animals, and boosting immune performance of the affected animals (Bower et al. 1994, Munday et al. 1997). Scuticociliates are difficult to treat once they invade host organisms, but are susceptible to a wide range of chemotherapeutants when they are outside the host (Novotny et al. 1996, Crosbie and Munday 1999).

The first scuticociliate infection reported from a crustacean was found by Cattaneo (1888) who described an *Anophrys*-like ciliate (=*Mesanophrys*, *Paranophrys*, see Armstrong et al. 1981, Sparkes et al. 1982, Small and Lynn 1985) in the hemolymph of European shore crabs (*Carcinus maenas*) in Italy, and later in France at a prevalence of 0.2% (Poisson 1930). Since that time many other instances of infection of crustaceans by endoparasitic ciliates have been reported in the scientific literature. Some of these include an *Anophrys*-like ciliate which was found by Bang et al. (1972) in "overwhelming numbers" in the haemolymph of moribund and dying edible crabs (*Cancer pagurus*) held in holding tanks in Brittany, France. Infection lead to opacity of the haemolymph and elevated mortalities in the captive stock. Death

ensued within a few days of crabs entering the holding facilities, and the infection could be passaged by the injection of infected haemolymph into naïve crabs (Bang et al. 1972). Other reported instances of scuticociliate disease include a *Parauronema* sp. in the hemocoel of cultured juveniles of the penaeid *Penaeus aztecus* in the Gulf of Mexico (Couch 1978), and lethal infections of a *Paranophrys* sp. in the haemolymph of Dungness crabs (*Cancer magister*) held in a tanks in a laboratory in Oregon, USA (Armstrong et al. 1981). In the latter case, it was found that all moribund infected crabs (40% prevalence) had recent wounds to the exoskeleton which provided a portal of entry for the ciliates (45-61 x 4-6 µm in mean dimension), after which disease and mortality occurred within 9-26 days due to extensive tissue damage in a variety of organs, including the heart (Armstrong et al. 1981).

Sparkes et al. (1982) reported the pathology associated with systemic scuticociliate (*Paranophrys* sp.) infections in diseased wild and captive *C. magister* from the east coast of the USA. The ciliates apparently entered the crabs via wounds inflicted by predators or during capture, after which they multiplied and spread to all major organ systems via the haemolymph. Affected crabs also displayed a tendancy to autotomise legs (Sparkes et al. 1982). Persistence of problems with ciliate infections in captive *C. magister* along the east coast of the USA lead to the description of a new ciliate (27-72 x 8-16 µm in dimension) which was described as *Mesanophrys pugettensis* by Morado and Small (1994). Later studies by Morado et al. (1999) in the same region recorded wild *C. magister* dead and dying during their moult from natural *M. pugettensis* infections along the coast of Washington state. Around the same time, surveys of blue crabs (*Callinectes sapidus*) in the same region by Messick and Small (1996) reported infections by another new ciliate *Mesanophrys chesapeakensis* (28-47 x 11-18 µms in dimension) which was found in 0.4-0.8% of wild blue crabs from Chesapeake Bay, mainly in the winter months (Messick 1998).

Several years later *Orchitophrya stellarum*, a parasitic ciliate originally reported infecting sea stars from Japan, Europe and North America (Byrne et al. 1997) was found causing disease in blue crabs (*Callinectes sapidus*) being held at research facilities during the winter months at the Virginia Institute of Marine Science (Small et al. 2013. Miller et al. 2013). Trials conducted by Miller et al. (2013) found that *O. stellarum* can find injured hosts using chemotaxis, but required a portal of entry for successful host invasion as it preferentially infected crabs with autotomised limbs. Infection via inoculation into the bloodstream could be successfully achieved with doses as little as 10 ciliates per crab, while high intensity infections developed quickly at doses over 500 ciliates per crab at 10–15°C, but not at 23°C, with crabs infected with *O. stellarum* showing high levels of autotomy of periopods (Miller et al. 2013). It appears likely, based on molecular evidence, that *O. stellarum* (or a closely related species of *Mesanophrys*) has also been reported causing disease and mortalities of up to 80% in swimming crabs (*Portunus trituberculatus*) cultured in China at 12-15°C (Liu et al. 2020). Most recently, Metz and Hechinger (2021) erected a new genus *Lynnia* to describe a scuticociliate *Lynnia grapsolytica* which they found causing disease and mortality wild grapsid crabs *Pachygrapsus crassipes* in California. Observations of wild-caught crabs in captivity found that *L. grapsolytica* raises the overall death rate of affected crab populations by 13 – 22%, and infection caused experimental crabs to die at a 2.6x greater daily rate than uninfected crabs (Metz and Hechinger 2021).

Scuticociliate infections have also been problematic in the captive holding of lobsters. For example, the scuticociliate *Anophryoides haemophilia* infects wild and captive American lobsters (*Homarus americanus*) from the east coast of North America causing "bumper car" disease mainly during the

autumn and winter months (Aiken et al. 1973, Cawthron et al. 1996, Lavallée et al. 2001, Greenwood et al. 2005). This disease agent was found at low prevalences of (0.4%) in wild *H. americanus*, but was responsible for occasional mass mortalities and significant (10-15%) pre-processing mortality in lobsters held in onshore holding facilities (Lavallée et al. 2001, Greenwood et al. 2005). Experimental infections by Athanassopoulo et al. (2004) found that *A. haemophila* was lethal to captive lobsters which, depending on initial dose rate, showed pathological lesions in gills and myocardium between 4 and 9 weeks post-infection at water temperatures of 2°C. Experimental lobsters died between weeks 4 and 6 post-infection when inoculated with 500,000 ciliates, while lobsters inoculated with 2000 ciliates died within 11-14 weeks of infection (Athanassopoulo et al. 2004). Small et al. (2005a, 2005b) also reported the presence of a histophagous ciliate infection in the Norway lobster (*Nephrops norvegicus*) captured from the wild in Scotland. The ciliate was 35-65 µm x 12–26 µm in dimension and morphologically similar to scuticociliates in the genus *Mesanophrys*, but molecular analysis found it was genetically related more closely to *Orchitophyra stellarum*. The two lobsters infected displayed hemocytopenia, degeneration and necrosis of the heart muscle, and extensive infiltration of many organs particularly the gills (Small et al. 2005a).

In Australia, the scuticociliate *Uronema nigricans* was reported to cause disease and mortality in captive southern bluefin tuna (*Thynnus maccoyii*) held in sea cages near Port Lincoln in South Australia (Munday et al. 1997). Death of infected tuna was due to encephalitis after affected fish were compromised by low water temperatures and poor water quality (Munday et al. 1997, Crosbie and Munday 1999). *Uronema*-like ciliates were also responsible for encephalitis and mass mortalities in captive barramundi cod (*Chromileptes altivelis*) held in recirculated seawater (28°C, 32‰ salinity) in a landbased aquaculture system in QLD in June 2007 (Diggles 2011, B.K. Diggles and M. Landos, personal observations). In freshwater, infections by *Tetrahymena* spp. and *Chilodonella* spp. have caused disease in many species of captive finfish including golden perch (*Macquaria ambigua*), silver perch (*Bidyanus bidyanus*), Murray cod (*Maccullochella peeli*) and barramundi (*Lates calcarifer*) (Rowland and Ingram 1991, O'Donoghue and Adlard 2000, Herbert and Graham 2008, Diggles 2011, Bastos Gomes et al. 2017). In crustaceans *Tetrahymena pyriformis* caused lethal systemic infections in red-clawed crayfish (*Cherax quadricarinatus*) cultured in north QLD (Edgerton et al. 1995, 1996, 2002). The disease affected around 9% of moribund crayfish from one farm, with affected crayfish exhibiting lethargy and loss of righting reflex. Massive numbers of *T. pyriformis* (30-75 µm x 20-50 µm in dimension) were observed in a systemic infection of all major organs including the connective tissues of the hepatopancreas, the heart and the haemal sinuses of the gills, with little evidence of a host response (Edgerton et al. 1995, 1996). The ciliates were considered opportunistic invaders of compromised crayfish which had injuries such as carapace abrasions, puncture wounds or missing periopods (Edgerton et al. 1996).

5.7.5 Release assessment

Scuticociliate infections have not been recorded from lobsters or crabs in Australia to date, however it is known that scuticociliates occur naturally in the Australian environment and can infect a wide range of fish and shellfish species that occur in marine waters around the country (Munday et al. 1997, Diggles 2011). The prevalence and intensity of natural scuticociliate infections in wild commercially important species of crabs and lobsters in the northern hemisphere appears to be generally low, usually ranging between 0.2-0.5% and seasonally up to 0.8% (Poisson 1930, Messick 1988, Morado et al. 1999, Lavallée et al. 2001). However, it appears that the prevalence of scuticociliate agents can be much higher in

commercially important crustacean populations at certain locations (see Morado et al. 1999), while other species of non-commercially important crabs can exhibit much higher natural prevalences in the range of 10-30% (Morado and Small 1994, Metz and Hechinger 2021). Even though the disease status of TRL in northern Australia is poorly described, these precedents from oveserseas suggest that the likelihood of wild caught adult TRL collected for broodstock being infected by scuticociliates is non-negligible.

Different species or strains of marine scuticociliates may exist in different parts of the country (Bastos Gomes et al. 2017), although their distribution is not known at this time. There is little information published in relation to host specificity of scuticociliates capable of colonising crustaceans, although host specificity may be low based on the apparent wide known host range for some species (Messick and Small 1996, Byrne et al. 1997, Small et al. 2005a, Miller et al. 2013, Liu et al. 2020), as well as the data of Miller et al. (2013) who found that *O. stellarum* from blue crabs (*C. sapidus*) could also successfully infect fiddler crabs (*Uca minax*). In the wild, however, the ability of scuticociliates to naturally infect and persist in populations of crustacean hosts is probably related to environmental conditions that determine the number of ciliates present in the water, the route of entry (i.e. availability of injured hosts) as well as the immune status of the host. This suggests that hatcheries and holding facilities situated in inshore regions of northern Australia would likely be exposed to scuticociliates vectored by wild populations of crustaceans and possibly even other hosts (finfish, echinoderms) via the intake water. Taking into account the information above, the risk of release is not negligible, and unrestricted likelihood estimations for the release of scuticociliates into northern Australian waters via the various release pathways are provided below.

Release assessment for Scuticociliate disease

Pathways	Via broodstock into hatchery in north QLD	Via water into hatchery in north QLD	Via juveniles into waters of QLD	Via juveniles into waters of the NT	Via juveniles into waters of WA
Likelihood of release	Moderate	High	Low	Low	Low

5.7.6 Exposure assessment

Crustaceans in marine and freshwater regions throughout northern Australia are already at risk of natural exposure to scuticociliates, as these parasites are free living opportunistic pathogens that are likely to be widespread in the natural environment. However with a few notable exceptions, infections of wild crustaceans with scuticociliates tend to be uncommon, probably due to the relative lack of eutrophic conditions and limited availability of compromised hosts. Indeed, the literature suggests that disease caused by scuticociliates mainly occurs in circumstances where crabs or lobsters are held in captivity at high densities in nutrient enriched water typical of commercial holding or aquaculture rearing environments (Bang et al. 1972). Even under these circumstances, infection of new hosts only tends to occur if a portal of entry is provided, such as when crustaceans are damaged by predators or rough handling during capture, resulting in puncture wounds or loss of periopods (Armstrong et al. 1981, Sparkes et al. 1982, Miller et al. 2013). On the other hand, perforations of the exoskeleton need not be large, as scuticociliates are small (typically around 50 x 15 µm in dimension) and can find injured hosts using chemotaxis (Miller et al. 2013).

Successful infection and establishment of scuticociliates in new hosts will also occur only if sufficient quantities of scuticociliates (i.e. an infective dose) are introduced into the water surrounding a damaged animal under conditions suitable for transmission. The quantities of scuticociliates required to successfully transmit infection horizontally via injection of viable ciliates has been determined in some instances. For example, Athanassopoulo et al. (2004) found that American lobsters (*Homarus americanus*) held in cold (2°C) seawater became diseased and died within 11-14 weeks of being infected with 2000 *Anophryoides haemophila*. The replication rate of ciliates and hence the course of infection is likely to be much faster at higher water temperatures, as shown by the study of Miller et al. (2013) who found the minimum infective dose (10-100 ciliates) of *O. stellarum* resulted in mortality of 10-25% of fiddler crabs (*U. minax*) after 13 days at 10–15°C. On the other hand, *O. stellarum* did not successfully transmit infection at 23°C, possibly due to the latter temperature being higher than the optimal range found for growth and survival of that parasite (Small et al. 2013, Miller et al. 2013). Intensities of *O. stellarum* in the haemolymph of moribund blue crabs and fiddler crabs often exceeded 1x 10^6 ciliates per ml of haemolymph, suggesting that 1 ml of infected haemolymph contained enough parasites to infect around 10,000 other crabs via the inoculation route (Miller et al. 2013).

It is clear that scuticociliates which occur in the Australian environment could infect broodstock lobsters if they were injured during handling, and could also enter holding facilities and hatcheries via intake water. Furthermore, they could also be horizontally transmitted to captive juvenile lobsters within the hatchery environment in the absence of appropriate biosecurity precautions, and environmental conditions are likely to be suitable for disease transmission in the wild in northern Australia. Taking these various factors into consideration, the risk of exposure and establishment is non-negligible, and the overall likelihood of exposure and establishment of scuticociliates is considered to be **Moderate**.

5.7.7 Consequence assessment

Scuticociliates are already likely to be already present in northern Australia. These parasites are also facultative pathogens and there is limited evidence that scuticociliates can cause major disease outbreaks in wild crustaceans, though it appears that they can do so in some hosts (Metz and Hechinger 2021) particularly in situations when environmental conditions are suitable (Morado et al. 1999). However, scuticociliates have been proven many times to be significant pathogens of captive crustaceans held at high densities, including captive lobsters in which they can cause significant mortalities and economic damage (Cawthron et al. 1996, Greenwood et al. 2005). The translocation of these parasites with culturesd TRL could therefore have significant impacts on captive lobster populations.

On the other hand, scuticociliate infection is not listed by the OIE or NACA, nor is this disease listed as reportable in any jurisdictions within Australia. Hence the spread of scuticociliates into new areas is unlikely to adversely impact trade. These disease agents are probably already present in many locations in the wild, however little is known about the distribution and epizootiology of scuticociliate infections in Australian crustaceans, except for the fact to date there has been no evidence that these parasites have had any discernible impact on wild populations. Considering all of these factors, establishment of scuticociliates into new areas may have significant consequences for aquaculture of susceptible crustaceans (particularly crabs and lobsters), however given the facultative nature of the disease process and the fact that it represents a threat mainly to the health of TRL that are injured or otherwise compromised, if scuticociliates were translocated and established they may be amenable to control. Their introduction into new regions therefore is unlikely to cause significant biological consequences or

environmental effects, nor are they likely to inflict severe adverse economic consequences for crustacean fisheries. It is therefore estimated that the overall consequences of introduction and establishment of scuticociliates into the environment of northern Australia via the identified risk pathways would likely be **Low**.

5.7.8 Risk estimation

The unrestricted risk associated with scuticociliate disease is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The unrestricted risk estimate for scuticociliate disease exceeds the ALOP for some pathways, suggesting that additional risk management is required for these disease agents.

Risk estimate for Scuticociliate disease

Pathways	Via broodstock into hatchery in north QLD	Via water into hatchery in north QLD	Via juveniles into waters of QLD	Via juveniles into waters of the NT	Via juveniles into waters of WA
Combined likelihood of release and exposure	Low	Moderate	Low	Low	Low
Consequences of establishment	Low	Low	Low	Low	Low
Risk estimation	Very low risk 6	Low risk 8	Very low risk 6	Very low risk 6	Very low risk 6

6.0 Risk Mitigation

6.1. Risk Evaluation

The results from the risk assessment are summarised in Table 8 below. The risk assessment process identified that additional risk management is required for one or more pathways for 7 of the 8 identified diseases of concern. These included moderate to high risks of infection with white spot syndrome virus (WSSV), moderate risks of infection with undescribed endemic viruses, moderate to low risks of infection with haplosporidians and *Hematodinium* spp., and low risks of microsporidosis, infection with rickettsia like organisms which can cause milky haemolymph disease, and infection with scuticociliates. The pathways for which the unrestricted risk for release and establishment of at least one disease agent exceeded the ALOP included introduction of broodstock into the hatchery (5 out of 8 diseases), intake of water into the hatchery (7 of 8 diseases), and translocation of juvenile TRL from the hatchery into sea rafts for grow out within the waters of QLD, NT or WA (6 of the 8 diseases).

Table 8. Summary table for unrestricted risk estimate outcomes from the risk assessment.

Pathway	Via broodstock into hatchery in north QLD	Via water into hatchery in north QLD	Via juveniles into waters of QLD	Via juveniles into waters of the NT	Via juveniles into waters of WA
Viruses					
Infection with *Panulirus argus* virus 1 (PaV1)	Very low risk 4	Very low risk 4	Very low risk 4	Very low risk 4	Very low risk 4
Infection with undescribed endemic viruses	Moderate risk 12	Moderate risk 12	Moderate risk 12	Moderate risk 12	Moderate risk 12
Infection with white spot syndrome virus (WSSV)	Moderate risk 12	High risk 16	Moderate risk 12	Moderate risk 12	Moderate risk 12
Bacteria					
Milky haemolymph disease of spiny lobsters (MHD-SL) (or similar RLO)	Low risk 8	Low risk 8	Low risk 8	Low risk 8	Low risk 8
Fungi					
Microsporidosis	Very low risk 6	Low risk 9	Low risk 9	Low risk 9	Low risk 9
Protozoa					
Haplosporidosis	Low risk 8	Moderate risk 12	Low risk 8	Low risk 8	Low risk 8
Infection with *Hematodinium* spp.	Low risk 9	Moderate risk 12	Low risk 9	Low risk 9	Low risk 9
Scuticociliate disease	Very low risk 6	Low risk 8	Very low risk 6	Very low risk 6	Very low risk 6

Only one of the diseases of concern, namely infection with PaV1, did not exceed the ALOP at this time. This was because the risk assessment found that while the consequences of establishment of PaV1 virus in TRL hatcheries and wild populations of crustaceans in northern Australia would be high, the fact that this virus is exotic to Australia meant that the risk of its release via various pathways ranged from

extremely low to very low, such that the overall risk for all pathways examined remained within the ALOP. However, the risk of release of PaV1 into Australian waters was not negligible due to the existence of frozen lobster (*Panulirus argus*) tails in retail supermarkets which are sourced from regions where PaV1 is known to occur. Given that it is well known that use of imported seafood products in Australia as bait or burley by recreational fishers is widespread (Kewagama Research 2007, Future Fisheries Veterinary Service 2017, Senate 2017, Kantar Public 2019), a testing program is recommended for lobster tails imported into Australia to determine what proportion of frozen uncooked *P. argus* tails available for retail sale in supermarkets in Australia are positive for PaV1. In addition, research is recommended in order to determine if TRL native to Australia are susceptible to infection with PaV1. For the remaining 7 disease agents of concern, options for risk mitigation to reduce the risks of their translocation via the various pathways are considered in more detail in Section 6.2.

6.2 Options for risk mitigation

This section identifies potential risk mitigation measures which may be able to reduce the risk estimate for each pathway and disease of concern back to within the ALOP (i.e. to reduce the risk to a probability of occurrence less frequent than 1 in 100 years). These risk mitigation methods should form the basis of biosecurity protocols and standard operating procedures during collection of broodstock, operation of the hatchery facility and translocation of juvenile TRL from the hatchery into sea rafts for grow out in QLD, NT and WA in order to reduce the risk of translocation of diseases of concern to within the ALOP.

The risk mitigation processes examined in this section of the RA relate only to option evaluation, together with an appraisal of the utility of each option for reducing risks to within the ALOP. These options could then form the basis of a consultation process that engages Government and stakeholders to evaluate the biosecurity risks involved with unrestricted pathways/mechanisms/risk factors for entry with a view towards identifying practical mitigation options that would reduce the risks identified to an acceptable level. The final risk management methods chosen will need to take into account a wide variety of pathway, disease, industry and region-related factors.

However, before discussing options for risk mitigation, the lowest risk option should be mentioned first, this being avoidance of translocation. At all times it must be realized that in the vast majority of circumstances use of local broodstock spawned in a local hatchery to produce spat to stock local waterways will be the “least risk option”. This is because evaluation of the risks involved with proposed translocations is always undertaken with imperfect knowledge of the disease status of the source jurisdictions, especially in this case due to the very limited amount of active disease surveillance of TRL populations in Australia at this time. This imperfect knowledge can affect the outcomes of the risk assessment process. For example, the apparent absence of a pathogen in a region or TRL population may be due to a lack of surveillance, which would then lead to relatively low risk estimates during a risk assessment process. Because of this, a cautious approach is recommended that embraces risk mitigation at every step of the TRL production cycle.

6.2.1 Pathway 1. Broodstock: Released into the hatchery in north QLD

Disease agent	Unrestricted Risk Estimate
MHD-SL (or similar endemic RLO) Haplosporidosis Infection with *Hematodinium* spp.	Low risk
Undescribed endemic viruses White spot syndrome virus (WSSV)	Moderate risk

The movement of live adult *P. ornatus* broodstock into the hatchery at Toomulla Beach, QLD, particularly those wild caught *P. ornatus* captured from the waters around Mackay, Cairns and Townsville, was found to pose a low risk of introduction of MHD-SL (or a similar endemic RLO), haplosporidosis and infection with *Hematodinium* spp., and a moderate risk of introduction of undescribed endemic viruses and white spot syndrome virus (WSSV). Furthermore, as noted in Sections 3.1 and 5.7 of this document, there are also several other less significant diseases which could also be harboured by broodstock lobsters. It is also worth noting here that the risk of introduction of all these diseases via *P. ornatus* broodstock translocated into the hatchery from the University of Tasmania IMAS facility is likely be lower than for wild caught broodstock, due to the long history of captive domestication of the IMAS stock.

Low risk disease agents

Risk of introduction of MHD-SL (or a similar endemic RLO) would be significantly reduced by ensuring that *P. ornatus* broodstock are not fed trash fish, molluscs and particularly decapod crustaceans. Instead, it is strongly recommended that *P. ornatus* broodstock are fed a nutritionally complete formulated diet. If supplementation with natural feeds is required for conditioning purposes etc., these should be restricted to non-crustacean sources (fish, molluscs) only and all natural feeds should be sanitised in some way via heat treatment (minimum 70°C for 5 minutes, or 100°C >1 minute) or 50 kilogray (kGy) gamma irradiation, or some other equivalent method to ensure that any pathogenic disease agents that may be present in natural feeds are inactivated. *Hematodinium* spp. are usually prevalent during the summer and autumn months (Messick and Sheilds 2000, Frischer et al. 2006, Small et al. 2019a), while haplosporidians are also known to infect wild jelly prawns in inshore areas of SE QLD during late summer and early autumn (Diggles et al. submitted). This suggests that collection of broodstock *P. ornatus* should occur only from pristine offshore environments, preferably during the cooler months of the year. The risk of introduction of RLOs, haplosporidians and *Hematodinium* spp. into the broodstock population would also be reduced by minimising the number of broodstock collected and bought into quarantine, and testing samples of haemolymph from each broodstock lobster for *Hematodinium* spp., RLOs and haplosporidians by PCR using specific primer sets (Small et al. 2007, OIE 2008, Hartikainen et al. 2014) to ensure they are free from infection prior to releasing them from quarantine.

Hematodinium spp. and RLOs have direct lifecycles and could be easily transmitted within the hatchery environment. In contrast, the presumed multihost lifecycle of haplosporidians should reduce the risk of disease transmission within the hatchery, especially if broodstock TRL are not fed natural feeds (which could act as alternative hosts containing haplosporidians) and have no contact with any other potential alternative hosts. Even so, pseudo-vertical transmission of haplosporidians may be still possible via crustaceans contacting waterborne vegetative stages, so it still remains possible that haplosporidians could be transmitted in hatcheries if larvae or juvenile crustaceans were spawned from infected broodstock

(Utari et al. 2012) or held in water where haplosporidian vegetative stages have not been filtered out or inactivated. Because of these reasons, it is important that broodstock *P. ornatus* are completely isolated from all other *P. ornatus* and other invertebrates in the hatchery at all times both physically and spatially (in separate rooms and water supplies) as well as operationally (complete separation of all equipment used on broodstock to ensure it never leaves the broodstock room), and are held in water filtered to 1µm followed by UV irradiation to a dose of at least 94 mJ/cm^2 (see Fernandez-Boo et al. 2021) with decontamination of all effluent water. At the end of their working lives, necropsy and destructive testing of all retired broodstock for both these pathogens using histopathology and PCR, should also be undertaken to improve our understanding of the health status of TRL in northern Australia.

Moderate risk disease agents

WSSV is known to infect a wide range of host species, but to date the virus has only been detectable in hosts captured in inshore areas of SE QLD during the late summer and autumn months (DAF QLD 2017, Oakey and Smith 2018, Oakey et al. 2019, Diggles 2020a, 2020c). The risk of introduction of white spot syndrome virus (WSSV) and also undescribed endemic viruses into the hatchery with *P. ornatus* broodstock could therefore be reduced if collection of broodstock occurred only from pristine offshore environments away from recreational fishing, preferably during the cooler months of the year. The risk of introduction of WSSV and undescribed endemic viruses into the broodstock population would also be reduced by minimising the number of broodstock collected from the wild and bought into quarantine, while WSSV risk can be further mninimised by testing pleopods and/or samples of haemolymph from each broodstock lobster for WSSV by qPCR (OIE 2021b) to ensure they are free from infection prior to releasing them from quarantine.

WSSV is effectively transmitted via the per-os route, hence it is strongly recommended that *P. ornatus* broodstock are fed a nutritionally complete formulated diet. If supplementation with natural feeds is required for conditioning purposes etc., these should be restricted to non-crustacean sources (fish, molluscs) only and these natural feeds should be sanitised in some way via heat treatment (minimum 70°C for 5 minutes, or 100°C >1 minute) or 50 kilogray (kGy) gamma irradiation, or some other equivalent method to ensure that WSSV or any other pathogenic disease agents that may be present in natural feeds are inactivated. Finally, given that these viruses can also be transferred horizontally through the water, broodstock TRL should be held completely isolated from all other *P. ornatus* in the hatchery at all times in water filtered to 1µm followed by UV irradiation to a dose of > 250 mJ/cm^2 (Chang et al. 1998b, Balasubramanian et al. 2006) and/or ozonation (5 mg/L/min, see Chang et al. 1998b and Section 6.2.2 for more details). Destructive testing of retired broodstock at the end of their working lives using histopathology to allow examination for WSSV and other viral inclusions which may indicate the presence of unknown endemic viruses, as well as testing for WSSV via qPCR should also be undertaken to improve our understanding of the health status of TRL in northern Australia.

Other disease agents

As noted in Section 3.1, broodstock lobsters may also experience infections of brooded egg masses by fungi, water moulds, and rhizocephalan barnacles. The potential presence and impact of these agents on fecundity and survival of eggs should be noted during development of broodstock husbandry protocols. Finally, if broodstock lobsters are damaged during capture or handling, this can provide a portal of entry for scuticociliates or ubiquitous marine bacteria such as *Vibrio* spp., *Aquimarina* sp and other

chitinoclastic bacteria responsible for shell disease and tail fan necrosis. Hence care should always be taken to ensure that broodstock lobsters are handled gently at all times so to minimize the risk of autotomy of periopods, damage to the carapace or punctures of the arthrodial membranes, all of which could provide portals of entry for opportunistic disease agents.

6.2.2 Pathway 2. Water: Taken into the hatchery in north QLD

Disease agent	Unrestricted Risk Estimate
MHD-SL (or similar endemic RLO) Microsporidosis Scuticociliate disease	Low risk
Haplosporidosis Infection with *Hematodinium* spp Undescribed endemic viruses	Moderate risk
White spot syndrome virus (WSSV)	High Risk

The intake of untreated water into the hatchery at Toomulla Beach, QLD, was found to pose a low risk of introduction of MHD-SL (or a similar endemic RLO), microsporidosis, and scuticociliate disease, a moderate risk of introduction of haplosporidosis, infection with *Hematodinium* spp., and undescribed endemic viruses, and a high risk of introduction of white spot syndrome virus (WSSV).

Low risk disease agents

The risk of introduction of MHD-SL (or a similar endemic RLO), microsporidosis and scuticociliates into the hatchery via untreated intake water was considered to exceed the ALOP due to the high likelihood that the inshore waters of north QLD contain many potential hosts and vectors for these disease agents. Treatment of intake water by mechanical filtration is unlikely to be sufficient on its own to reduce this risk to an acceptable level. This is because while scuticociliates are relatively easy to exclude due to their relatively large size (typically 30-70 µm x 10–30 µm in dimension), RLOs and microsporidian spores are much smaller, typically in the range of 1.4-2.0 µm x 0.6 µm in the case of RLOs (OIE 2008) and 2.0-2.4 µm x 1.4-1.8 µm for microsporidian spores (Dennis and Munday 1994). For these reasons, mechanical filtration to 1 µm may not provide sufficient protection, hence disinfection of intake water with UV irradiation and/or ozonation is recommended to ensure that all of these organisms are inactivated (Liltved and Cripps 1999, Summerfelt 2003). Disinfection of culture water by UV irradiation is widely used in aquaculture, with inactivation of the target microorganisms occurring due to denaturing of their DNA (Summerfelt 2003). Effective UV disinfection of water requires mechanical filtration of water prior to UV treatment to prevent light shadowing and maximise UV transmittance through the water (Liltved and Cripps 1999, Summerfelt 2003). Total microbicidal UV dosage is usually calculated in millijoules/cm² based on the relationship of 1 mJ/cm² = 10J /m²= 1,000 µW/cm² per second, i.e.

$$\frac{\text{total dose in mJ/cm}^2 = \text{intensity } (\mu\text{W/cm}^2) \times \text{duration of exposure (sec)}}{1000}$$

A typical recommendation for marine hatcheries specifies mechanical filtration down to 50 or 20 µm by passing intake water through drum or sand filters, followed by bag or cartridge filters down to 1 µm, then exposing the water to a minimum UV dose of around 30 mJ/cm² up to a maximum dose of 500-900 mJ/cm² depending on the pathogens requiring inactivation (Chang et al. 1998b, Kasai et al. 2002b). UV

doses of around 20-30 mJ/cm^2 are sufficient to inactivate many types of bacteria and some microsporidians (Kasai et al. 2002b, Chevrefils et al. 2006, Kent et al. 2009). However, a UV dose of 200 mJ/cm^2 is required to inactivate scuticociliates (Kasai et al. 2002a).

Ozone (O_3) is a highly reactive oxidative molecule that inactivates a wide range of microorganisms by damaging membranes and cell walls (Summerfelt 2003, Powell and Scolding 2018). Its application to seawater requires ozone generation, ozone transfer and contact time in solution, followed by ozone destruction to ensure that minimal residual oxidants are introduced into tanks containing cultured aquatic animals (Summerfelt 2003). Total ozone dose (*Ct)* is usually calculated in milligrams of total residual oxidants (TRO) per litre of water treated per unit time of exposure (mg/L/min) i.e:

Total Ozone Dose (*Ct*) = TRO (mg/L) x duration of exposure (in min)

A typical recommendation for ozone dose to inactivate aquatic pathogens in intake water is 0.5 mg/L residual oxidant concentration for 15-60 seconds (total dose 0.125-0.5 mg/L/min), though scuticociliates require 0.8 mg/L for 1 minute (total dose 0.8 mg/L/min, see Kasai et al. 2002b), and microsporidians may require doses similar to scuticociliates (Jacangelo et al. 2002, John et al. 2005). Care is needed to ensure accuracy and consistency when measuring ozone dose in seawater, especially when using oxidation/ reduction potential (ORP) probes which measure in millivolts (mV), as the relationship between mV and TRO at *Ct* > 1 mg/L is indirect and non-linear (Buchan et al. 2005, Powell and Scolding 2018).

Moderate risk disease agents

The risk of introduction of Haplosporidosis, *Hematodinium* spp. and undescribed endemic viruses into the hatchery via intake water was considered to exceed the ALOP due to the high likelihood that the inshore waters of north QLD harbour many potential hosts and vectors for these disease agents. Treatment of intake water by mechanical filtration down to 1 µm should eliminate dinospores of *Hematodinium* spp. which range in size beween 6 -17 µm (Li et al. 2011b). However, it is unlikely to be sufficient on its own to eliminate endemic viruses (which may be <1 µm in size), while the unknown infective stages of haplosporidians can pass through a 1 mm filter (Sunila et al. 2000), and a 150 µm filter, but not a 1 µm filter followed by UV irradiation at a dose of 30 mJ/cm^2 (Ford et al. 2001). Disinfection of intake water with UV irradiation and/or ozonation (Liltved and Cripps 1999, Summerfelt 2003) is therefore recommended to ensure that all of these organisms are inactivated. Following filtration to 1 µm to maximise UV transmittance, UV irradiation to a dose of at least 94 mJ/cm^2 may be required to ensure inactivation of all stages of haplosporidians (see Fernandez-Boo et al. 2021). However, if UV alone is used a higher dose is likely be required to inactivate *Hematodinium* spp., as dinoflagellates are generally inactivated between 100-150 mJ/cm^2 (Siemens 2011). Furthermore, if the full range of endemic viruses are to be inactivated with high certainty, an even higher UV dose may be required between 150-250 mJ/ cm^2 (Kasai et al. 2002b, Chevrefils et al. 2006, Liltved et al. 2006). If ozonation is used against haplosporidians, *Hematodinium* spp. and undescribed endemic viruses in intake water, an ozone dose of 0.5 mg/L for 4 minutes (2 mg/L/min) is usually sufficient to inactivate most viral and protozoan pathogens of aquatic animals (Kasai et al. 2002b, Liltved et al. 2006).

High risk disease agents

The risk of introduction of WSSV into the hatchery via intake water was considered to exceed the ALOP due to the fact that the inshore waters of north QLD harbour many potential hosts and vectors for WSSV (Diggles 2020c), recreational fishers continue to use imported frozen prawns as bait (and will do so for as long as these remain available for retail sale in supermarkets, see Kantar Public 2019), and there are no

natural barriers preventing movements of water or natural migration of wild crustaceans out of the Moreton Bay White Spot Biosecurity Area. Mechanical filtration of intake water will not prevent entry of WSSV which is <1 µm in size, hence disinfection of intake water with UV irradiation and/or ozonation (Liltved and Cripps 1999, Summerfelt 2003) is recommended. Over the years a range of UV doses with nearly 2 orders of magnitude difference [between 10-30 mJ/cm^2 (Nakano et al. 1998, Oseko et al. 2006) and 921 mJ/cm^2 (Chang et al. 1998b)] have been reported to effectively inactivate WSSV. This may be due to differences in methodology, initial viral dose studied or the susceptibility of hosts used in bioassays to determine virus viability post-treatment. Although it is notable that Chang et al. (1998b) found 10% of experimental prawns could still be infected by WSSV exposed to a UV dose of 461 mJ/cm^2, here it will be recommended that a "middle of the road" UV dose of > 250 mJ/cm^2 is required based on the study of Balasubramanian et al. (2006) who found UV at 150 mJ/cm^2 to be marginally insufficient, but 307 mJ/cm^2 to be enough to completely inactivate WSSV. If ozonation is to be used to inactivate WSSV in intake water, Chang et al. (1998b) found that a dose of 0.5 mg/L for 10 minutes (5 mg/L/min) was required to ensure complete inactivation of this virus.

6.2.3 Pathway 3. Juveniles: Released into the waters of QLD

Disease agent	Unrestricted Risk Estimate
MHD-SL (or similar endemic RLO) Microsporidosis Haplosporidosis Infection with *Hematodinium* spp.	Low risk
Undescribed endemic viruses White spot syndrome virus (WSSV)	Moderate risk

The translocation of juvenile TRL from the hatchery into sea rafts for grow out in QLD, was found to pose a low risk of introduction of MHD-SL (or a similar endemic RLO), microsporidosis, haplosporidosis and infection with *Hematodinium* spp., and a moderate risk of introduction of undescribed endemic viruses and white spot syndrome virus (WSSV).

Low risk disease agents

The risk of introduction of RLOs, microsporidosis, haplosporidosis and *Hematodinium* spp. via translocation of juvenile TRLs was considered to exceed the ALOP due to the non-negligible risk of infection of broodstock *P. ornatus* collected from the wild with these agents, and through infection of broodstock via use of natural feeds (see Section 6.2.1), combined with the potential for spread of all of these disease agents between cohorts of lobsters within the confined hatchery environment in the absence of effective biosecurity protocols. It is therefore important that each batch of larvae are removed from spawning tanks/vessels containing broodstock lobsters as soon as possible after eggs hatch and the larvae should remain completely isolated from all other invertebrates in the hatchery at all times both physically and spatially (in separate rooms and water supplies) as well as operationally (complete separation of all equipment used on larvae to ensure it never leaves the larval rearing room), with full decontamination of all effluent water. Cultures of live hatchery feeds should be regularly screened for these disease agents to ensure they do not vector them into larval lobsters. Upon weaning from live hatchery feeds larval and juvenile lobsters should be fed a complete formulated diet and subjected to regular health checks, whilst any unusual mortalities should be investigated using histopathology and by PCR to determine if RLOs,

microsporidians, haplosporidians and *Hematodinium* spp. are present using specific primer sets (Small et al. 2007, OIE 2008, Hartikainen et al. 2014, Stentiford et al. 2018). Juvenile TRL should also be subjected to statistically relevant batch testing using histopathology and PCR using specific primer sets to detect RLOs, microsporidians, haplosporidians and *Hematodinium* spp. prior to them being approved by the biosecurity authority in the relevant jurisdiction for translocation into sea rafts for grow out. Any batches of larvae or juveniles that subsequently test positive for any of these disease agents should be immediately destroyed and all exposed tanks and equipment thoroughly decontaminated and dried out. Any batches which do not suffer mass mortalities and subsequently test negative for these disease agents could be considered free from infection, therefore reducing the risk of their introduction into grow out rafts in QLD and from there into the environment to within the ALOP.

Moderate risk disease agents

The risk of introduction of WSSV and undescribed endemic viruses via translocation of juvenile TRLs was considered to exceed the ALOP due to the non-negligible risk of infection of broodstock *P. ornatus* collected from the wild with these agents, and through infection via use of natural feeds (see Section 6.2.1), combined with the potential for spread of both these disease agents between cohorts of lobsters within the confined hatchery environment as in the absence of effective biosecurity protocols. To reduce these risks each batch of larvae should be removed from spawning tanks/vessels containing broodstock lobsters as soon as possible after eggs hatch and the larvae should remain completely isolated from all other invertebrates in the hatchery at all times both physically and spatially (in separate rooms and water supplies) as well as operationally (complete separation of all equipment used on larvae to ensure it never leaves the larval rearing room), with full decontamination of all effluent water. Cultures of live hatchery feeds should be regularly screened for WSSV to ensure they do not act as vectors. Upon weaning from live hatchery feeds larval and juvenile lobsters should be fed a complete formulated diet and subjected to regular health checks, whilst any unusual mortalities should be investigated using histopathology for viral inclusions from undescribed endemic viruses and by qPCR (OIE 2021b) to determine if WSSV is present. Juvenile TRL should also be subjected to statistically relevant batch testing using histopathology and qPCR to detect these disease agents prior to them being approved by the biosecurity authority in the relevant jurisdiction for translocation into sea rafts for grow out. Any batches of larvae or juveniles that subsequently test positive for either of these disease agents should be immediately destroyed and all exposed tanks and equipment thoroughly decontaminated and dried out. Any batches which do not suffer mass mortalities and subsequently test negative for these disease agents could be considered free from infection, therefore reducing the risk of their introduction into grow out rafts in QLD and from there into the environment to within the ALOP.

6.2.4 Pathway 4. Juveniles: Released into the waters of NT

Disease agent	Unrestricted Risk Estimate
MHD-SL (or similar endemic RLO) Microsporidosis Haplosporidosis Infection with *Hematodinium* spp.	Low risk
Undescribed endemic viruses White spot syndrome virus (WSSV)	Moderate risk

Due to the paucity of information on the disease status of TRL throughout northern Australia at this time, there is no information on which to base assessments whether there are any significant differences in the disease status of TRL from the different state jurisdictions of QLD, NT and WA. Furthermore, while WSSV is known to be present in south east QLD in a range of crustacean species within the Moreton Bay White Spot Biosecurity Area (Diggles 2020c), a range of biosecurity requirements have been implemented to try to prevent the anthropogenic spread of the disease agent from the infected zone into other areas of QLD and Australia (DAF QLD 2017, Diggles 2020a). For these reasons, the disease status of north QLD is herein considered to be similar to the NT and WA in this respect, and therefore the risk mitigation measures recommended for this pathway are identical to those recommended in section 6.2.3.

6.2.5 Pathway 5. Juveniles: Released into the waters of WA

Disease agent	Unrestricted Risk Estimate
MHD-SL (or similar endemic RLO) Microsporidosis Haplosporidosis Infection with *Hematodinium* spp.	Low risk
Undescribed endemic viruses White spot syndrome virus (WSSV)	Moderate risk

As previously discussed in section 6.2.4, due to the paucity of information on the disease status of TRL throughout northern Australia at this time, the disease status of WA is herein considered to be similar to QLD and the NT in this respect, and therefore the risk mitigation measures recommended for this pathway are identical to those recommended in section 6.2.3 (and 6.2.4).

6.3 Summary of suggested risk mitigation methods

The range of risk mitigation methods discussed in Section 6.2 could be used to form the basis of biosecurity protocols and standard operating procedures for collection of adult *P. ornatus* broodstock from the waters around Mackay, Cairns and Townsville, operation of the hatchery facility at Toomulla Beach, QLD, and translocation of juvenile TRL into grow out rafts in QLD, NT and WA to reduce risks of translocating disease agents of concern to acceptable levels. These suggested risk mitigation methods can be summarised as follows (see also Table 9):

Broodstock collection in QLD

P. ornatus broodstock should:

1. Only be collected from pristine offshore environments, preferably during the cooler months of the year. Inshore environments where recreational fishing occurs and close proximity (<15 km) to international shipping ports should be avoided.

2. The minimum number of broodstock lobsters (minimum dictated by hatchery genetic requirements) should be collected and care should always be taken to ensure that broodstock lobsters are handled gently at all times to minimise the risk of autotomy of periopods, damage to the carapace or punctures of the arthrodial membranes. Individuals with infections of brooded egg masses by fungi, water moulds, and rhizocephalan barnacles should also be avoided.

Broodstock transport and holding in hatcheries

P. ornatus broodstock should:

3. Be maintained in the hatchery in water filtered to 1 µm followed by UV irradiation to a dose of at least 250 mJ/cm^2 (and/or 5 mg/L/min ozone) to completely inactivate infective stages of the various disease agents that may occur in the hatchery water supply.

4. Remain completely isolated from all other invertebrates (including other TRL) in the hatchery at all times both physically and spatially (in separate tanks, rooms and water supplies) as well as operationally (complete separation of all equipment used on broodstock to ensure it never leaves the broodstock room).

5. Samples of haemolymph and/or pleopods from each broodstock lobster should be tested for WSSV, haplosporidians, *Hematodinium* spp. and RLOs by cPCR or qPCR using specific primer sets (Small et al. 2007, OIE 2008, Hartikainen et al. 2014, OIE 2021b) to ensure they are free from infection prior to releasing them from quarantine.

6. Be fed a nutritionally complete formulated diet. If supplementation with natural feeds is required for conditioning purposes etc., these should be restricted to non-crustacean sources (fish, molluscs) only and be sanitised via heat treatment (minimum 70°C for 5 minutes, or 100°C >1 minute) or 50 kilogray (kGy) gamma irradiation, or some other equivalent method to ensure that any pathogenic disease agents that may be present in natural feeds are inactivated.

7. All effluent water should be decontaminated by pumping it through a treatment system that incorporates ultrafiltration, or chemical dosing (e.g. chlorine minimum dose 200 mg/L for 30 min) or ozonation (minimum 2 mg/L/min dose, preferably 5 mg/L/min ozone), or to evaporation ponds that are isolated from nearby water bodies, so that the hatchery does not load the adjacent aquatic environment with crustacean pathogens over time.

8. At the end of their working lives, necropsy and destructive testing of all retired broodstock for WSSV, other viral inclusions which may indicate the presence of unknown endemic viruses, haplosporidians, *Hematodinium* spp., microsporidians and RLOs using histopathology and PCR, should also be undertaken to improve our understanding of the health status of TRL in northern Australia.

Spawning lobster larvae

9. Each batch of larvae should be removed from spawning tanks/vessels containing broodstock lobsters as soon as possible after eggs hatch, and the larvae should remain completely isolated from all other invertebrates in the hatchery at all times both physically and spatially (in separate rooms and water supplies) as well as operationally (complete separation of all equipment used on larvae to ensure it never leaves the larval rearing room), with full decontamination of all effluent water.

Rearing and testing of larvae

10. Every batch of larvae should remain completely isolated from all other invertebrates (excluding live feeds) in the hatchery at all times both physically and spatially (in separate tanks, rooms and water supplies) as well as operationally (complete separation of all equipment used to ensure it never leaves the larval rearing room), in water filtered to 1 µm followed by UV irradiation to a dose of at least 250 mJ/cm^2 (or 5 mg/L/min ozone) with full decontamination of all effluent water.

11. Cultures of live hatchery feeds should be regularly screened for WSSV, RLOs, microsporidians, haplosporidians and *Hematodinium* spp. to ensure they do not vector these disease agents into larval lobsters.

12. Upon weaning from live hatchery feeds larval lobsters should be fed a complete formulated diet and subjected to regular health checks.

13. Any unusual mortalities in larvae should be investigated using histopathology and by PCR to determine if WSSV, RLOs, microsporidians, haplosporidians and *Hematodinium* spp. are present using specific primer sets (Small et al. 2007, OIE 2008, Hartikainen et al. 2014, Stentiford et al. 2018, OIE 2021b). Histopathology should pay particular attention to any viral inclusions which may indicate the presence of unknown endemic viruses.

14. Any batches of larvae that test positive for any of the diseases of concern should be immediately destroyed by autoclaving or fixation in Davidsons fixative or 10% seawater formalin and/or 90% ethanol then either disposed of after use (autoclaved larvae) or examined for pathogens (fixed larvae), and all exposed tanks and equipment thoroughly decontaminated and dried out.

Rearing and testing of juvenile TRL

15. Every batch of juvenile TRL should remain completely isolated from all other invertebrates in the hatchery at all times both physically and spatially (in separate tanks, rooms and water supplies) as well as operationally (complete separation of all equipment used to ensure it never leaves the larval rearing room), in water filtered to 1 µm followed by UV irradiation to a dose of at least 250 mJ/cm^2 (or 5 mg/L/min ozone) with full decontamination of all effluent water.

16. Juvenile TRL should be fed a complete formulated diet and subjected to regular health checks. Any unusual mortalities should be investigated using histopathology and by PCR to determine if WSSV, RLOs, microsporidians, haplosporidians and *Hematodinium* spp. are present using specific primer sets (Small et al. 2007, OIE 2008, Hartikainen et al. 2014, Stentiford et al. 2018, OIE 2021b). Histopathology should pay particular attention to any viral inclusions which may indicate the presence of unknown endemic viruses.

17. Prior to their translocation into sea rafts for grow out, a statistically relevant sample of juvenile TRL should be subjected to disease testing using histopathology and PCR using specific primer sets to detect WSSV, RLOs, microsporidians, haplosporidians and *Hematodinium* spp. (Small et al. 2007, OIE 2008, Hartikainen et al. 2014, Stentiford et al. 2018, OIE 2021b) prior to them being approved for translocation by the biosecurity authority in the relevant jurisdiction.

18. Any batches of juvenile TRL that test positive for any of the diseases of concern should be immediately destroyed by autoclaving or fixation in Davidsons fixative or 10% seawater formalin and/or 90% ethanol then either disposed of after use (autoclaved larvae) or examined for pathogens (fixed larvae), and all exposed tanks and equipment thoroughly decontaminated and dried out.

19. Any batches of juvenile TRL which do not suffer unexplained mortalities and subsequently test negative for these disease agents could be considered free from infection, therefore reducing the risk of their introduction into sea rafts for grow out (and from there into the environment) in QLD, NT and WA to within the ALOP.

20. The hatchery providing juvenile TRL should be required to maintain a comprehensive hatchery biosecurity plan containing details of all relevant operational guidelines (including broodstock management, treatment of intake and effluent water, disinfection methods used, sampling for diagnostic testing etc.), and these documents should be audited by the relevant competent authority or an appropriately qualified third party on a regular (suggest annual) basis to ensure compliance with the audit requirements outlined in the generic guidelines for Aquaculture Farm Biosecurity Plans (Sub-Committee on Aquatic Animal Health 2017).

6.4 Update of RA

The threat from invasive pests and diseases continues to increase directly in line with increasing volumes of international trade (Diggles 2017b, 2020a, Scott-Orr et al. 2017). Given the fact that, for example, there are already known pathways via which lobster pathogens such as PaV1 virus could enter the Australian environment, it must be acknowledged that this risk analysis document represents a snapshot of the known disease situation at the time of publication. It will therefore need to be updated on a regular basis in the future as new information on diseases of TRL, including the status of PaV1 within Australia, becomes available.

Table 9. Summary of the various pathways upon which specific risk mitigation measures operate.

Proposed risk mitigation measures	Pathways			
	Broodstock source[1]	Hatchery water[2]	Hatchery environment[3]	Juveniles in sea rafts[4]
Broodstock collected from pristine offshore sites - not inshore areas or close to international shipping ports	✓		✓	✓
Minimum number of broodstock collected	✓		✓	✓
No broodstock with injuries or missing appendages	✓		✓	✓
Broodstock testing (haemolymph, pleopods, necropsy at end of working life)	✓		✓	✓
Disinfection of hatchery intake water (filtered to 1 µm, >250 mJ/cm^2 UV or 5 mg/L/min ozone)		✓	✓	✓
Decontamination of hatchery effluent water	✓	✓	✓	✓
Complete physical, spatial and operational separation between broodstock and larvae/juveniles. Removal of eggs from spawning tanks as soon as possible	✓		✓	✓
Broodstock and juveniles fed formulated diets	✓		✓	✓
Live hatchery feeds screened for pathogens			✓	✓
Mortality cutoff for larval and juvenile lobsters and diagnostic testing following any unusual mortalities			✓	✓
Lack of unusual mortalities, history of laboratory testing for reportable diseases and unusual mortality events	✓		✓	✓
Diagnostic batch testing of juveniles (histology and relevant PCRs) prior to translocation			✓	✓
Best practice hatchery protocols as per comprehensive hatchery biosecurity plan.	✓	✓	✓	✓

[1] Broodstock source = Reduce risk of pathogen being introduced into hatchery via broodstock.

[2] Hatchery water = Reduce risk of pathogen being introduced into hatchery via intake water

[3] Hatchery environment = Reduce risk of pathogen being introduced into juvenile production process via biosecurity breakdown within the hatchery.

[4] Juveniles in sea rafts = Reduce risk of pathogens being introduced into sea rafts during grow out.

7.0 Literature cited

Adl SM, and 47 other co authors (2019). Revisions to the classification, nomenclature, and diversity of eukaryotes. *Journal of Eukaryotic Microbiology* 66: 4-119.

Aiken DE, Sochasky JB, Wells PG (1973). Ciliate infestation of the blood of the lobster *Homarus americanus*. *International Council for the Exploration of the Sea* C.M. 1973/K:46

Amigo JM, Gracia MP, Rius M, Salvado H, Maillo PA, Vivares CP (1996). Longevity and effects of temperature on the viability and polar-tube extrusion of spores of *Glugea stephani*, a microsporidian parasite of commercial flatfish. *Parasitology Research* 82: 211-214.

Anderson IG, Shariff M, Nash G (1989). A hepatopancreatic microsporidian in pond-reared tiger shrimp, *Penaeus monodon*, from Malaysia. *Journal of Invertebrate Pathology* 53*:* 278-280.

Andrews JD (1968). Oyster mortality studies in Virginica. VII Review of epizootiology and origin of *Minchinia nelsoni*. *Proceedings of the National Shellfisheries Association* 58: 23-36.

Andrews JD (1982). Epizootiology of late summer and fall infections of oysters by *Haplosporidium nelsoni*, and comparison to annual life cycle of *Haplosporidium costalis*, a typical haplosporidian. *Journal of Shellfish Research* 2: 15-23.

Aranguren LF, Han JE, Tang KF (2017). *Enterocytozoon hepatopenaei* (EHP) is a risk factor for acute hepatopancreatic necrosis disease (AHPND) and septic hepatopancreatic necrosis (SHPN) in the Pacific white shrimp *Penaeus vannamei*. *Aquaculture* 471: 37–42.

Aranguren Caro LF, Mai HM, Nunan L, Lin J, Noble B, Dhar AK (2020). Assessment of transmission risk in WSSV-infected shrimp *Litopenaeus vannamei* upon cooking. *Journal of Fish Diseases* 43: 403-411.

Armstrong DA, Burreson EM, Sparks AK (1981). A ciliate infection (*Paranophrys* sp.) in laboratory-held Dungeness crabs, *Cancer magister*. *Journal of Invertebrate Pathology* 37: 201–209.

Arzul I, Carnegie RB (2015). New perspective on the haplosporidian parasites of molluscs. *Journal of Invertebrate Pathology* 131: 32–42.

Athanassopoulou F, Speare D, Cawthorn RJ, MacMillan R, Despres B (2004). Pathology of *Anophryoides haemophila* (Scuticociliatida: Orchitophryidae), parasite of American lobster *Homarus americanus* kept under experimental conditions. *Aquaculture* 236: 103–117.

Atherley NA, Freeman MA, Dennis MM (2020). Post-mortem examination of the Caribbean spiny lobster (*Panulirus argus*, Latreille 1804) and pathology in a fishery of the Lesser Antilles. *Journal of Invertebrate Pathology* 175: 107453

Austin B, Austin DA (2007). Bacterial fish pathogens: disease in farmed and wild fish. 4th edition. Springer. 552 pgs.

Azevedo C, Hine PM (2016). Haplosporidia. In: Archibald JM et al. (eds), Handbook of the Protists. Springer International Publishing, 29 pgs.

Bailey L (1972). The preservation of infective microsporidian spores. *Journal of Invertebrate Pathology* 20: 252–254.

Balasubramanian G, Sudhakaran R, Syed Musthaq S, Sarathi M, Sahul Hameed AS (2006). Studies on the inactivation of white spot syndrome virus of shrimp by physical and chemical treatments, and seaweed extracts tested in marine and freshwater animal models. *Journal of Fish Diseases* 29: 569-572.

Bang FB, Audouin J, Leglise M (1972). Ciliate infection of the blood of the edible crab, *Cancer pagurus*, in holding tanks in Brittany, France. *Journal of Invertebrate Pathology* 20: 226–227.

Barber RD, Ford SE (1992). Occurrence and significance of ingested haplosporidian spores in the eastern oyster, *Crassostrea virginica* (Gmelin, 1791). *Journal of Shellfish Research* 11: 371-375.

Bastos Gomes G, Miller TL, Vaughan DB, Jerry DR, McCowan C, Bradley TL, Hutson KS (2017). Evidence of multiple species of *Chilodonella* (Protozoa, Ciliophora) infecting Australian farmed freshwater fishes. *Veterinary Parasitology* 237: 8-16.

Bateman KS, Munro J, Uglow B, Small HJ, Stentiford GD (2012). Susceptibility of juvenile European lobster *Homarus gammarus* to shrimp products infected with high and low doses of white spot syndrome virus. *Diseases of Aquatic Organisms* 100: 169-184.

Bateman KS, Wiredu-Boakye D, Kerr R, Williams BA, Stentiford GD (2016). Single and multi-gene phylogeny of *Hepatospora* (Microsporidia) – a generalist pathogen of farmed and wild crustacean hosts. *Parasitology* 143: 971–982.

Baumgartner WA, Hawke1 JP, Bowles K, Varner PW, Hasson KW (2009). Primary diagnosis and surveillance of white spot syndrome virus in wild and farmed crawfish (*Procambarus clarkii, P. zonangulus*) in Louisiana, USA. *Diseases of Aquatic Organisms* 85: 15-22.

Bearham D, Spiers Z, Raidal S, Jones JB, Nicholls PK (2007). Molecular characterisation of a haplosporidian parasite infecting rock oysters *Saccostrea cucullata* in northern Western Australia. *Journal of Invertebrate Pathology* 95: 33–40.

Bearham D, Spiers Z, Raidal S, Jones JB, Nicholls PK (2008a). Detection of *Minchinia* sp. in rock oysters *Saccostrea cucullata* (Born 1778) using DNA probes. *Journal of Invertebrate Pathology* 97: 50-60.

Bearham D, Spiers Z, Raidal S, Jones JB, Burreson EM, Nicholls PK (2008b). Spore ornamentation of *Haplosporidium hinei* n. sp. (Haplosporidia) in pearl oysters *Pinctada maxima* (Jameson, 1901). *Parasitology* 135: 521–527.

Bearham D, Spiers Z, Raidal S, Jones JB, Nicholls PK (2008c). Spore ornamentation of *Minchinia occulta* n. sp. (Haplosporidia) in rock oysters *Saccostrea cucullata* (Born, 1778). *Parasitology* 135: 1271–1280.

Bearham D, Spiers Z, Raidal S, Jones JB, Nicholls PK (2009a). Detection of *Minchinia occulta* in samples of pearl oysters *Pinctada maxima* infected by *Haplosporidium hinei*. *Australian Veterinary Journal* 87: 430-437.

Bearham D, Raidal SR, Creeper J, Stephens F, Jones JB, McCallum B (2009b). Aquatic Animal Health Subprogram: Development of diagnostic tests to assess the impact of haplosporidian infections in pearl oysters. November 2009. FRDC Project 2006/064. 182 pgs. https://www.frdc.com.au/Archived-Reports/FRDC%20Projects/2006-064-DLD.pdf

Beckman CL, Hooper GE (2015). Blue crab (*Portunus armatus*) Fishery 2013/14. SARDI Research Report Series No. 837, April 2015. 70 pgs.

Behringer DC, Butler IM, Shields JD (2001). The first viral disease reported in lobsters. *The Lobster Newsletter* 14: July 2001, 1-3.

Behringer DC, Butler IM, Shields JD (2006). Avoidance of disease by social lobsters. *Nature* 441: 421.

Behringer DC, Butler MJ, Shields JD (2008). Ecological and physiological effects of PaV1 infection on the Caribbean spiny lobster (*Panulirus argus* Latreille). *Journal of Experimental Marine Biology and Ecology* 359: 26–33.

Behringer DC, Butler IV MJ, Shields JD, Moss J (2011). Review of *Panulirus argus* virus 1—a decade after its discovery. *Diseases of Aquatic Organisms* 94: 153–160.

Behringer DC, Butler IV MJ, Stentiford GD (2012a). Disease effects on lobster fisheries, ecology, and culture: overview of DAO special 6. *Diseases of Aquatic Organisms* 100: 89–93.

Behringer DC, Moss J, Shields JD, Butler MJ (2012b). PaV1 infection in the Florida spiny lobster fishery and its effects on trap function and disease transmission. *Canadian Journal of Fisheries and Aquatic Sciences* 69: 136−144.

Biju N, Sathiyaraj G, Raj M, Shanmugam V, Baskaran B, Govindan U, Kumaresan G, Kasthuriraju KK, Chellamma TS. (2016). High prevalence of *Enterocytozoon hepatopenaei* in shrimps *Penaeus monodon* and *Litopenaeus vannamei* sampled from slow growth ponds in India. *Diseases of Aquatic Organisms* 120: 225- 230.

Biosecurity Australia (2009). *Generic Import Risk Analysis Report for Prawns and Prawn Products*. Final Report. Biosecurity Australia, Canberra, Australia. 7 October 2009, 292 pgs.

Bobes R, Diaz J, Diaz E (1988). Aislamiento e identificacion de *Aerococcus viridans* var. *homari* en la langosta *Panulirus argus* con sintomas de septicemia. *Revista de Investigacion Marina* 9: 97–103.

Boschma H (1955). The described species of the family Sacculinidae. *Zoologische Verhandelingen* 27: 1-76.

Bourne DG, Young N, Webster N, Payne M, Salmon M, Demel S, Hall M (2004). Microbial community dynamics in a larval aquaculture system of the tropical rock lobster, *Panulirus ornatus*. *Aquaculture* 242: 31–51.

Bourne DG, Høj L, Webster NS, Swan J, Hall MR (2006). Biofilm development within a larval rearing tank of the tropical rock lobster, *Panulirus ornatus*. *Aquaculture* 260: 27–38.

Bower SM., Meyer GR (2002). Morphology and ultrastructure of a protistan pathogen in the haemolymph of shrimp (*Pandalus* spp.) in the northeastern Pacific Ocean. *Canadian Journal of Zoology* 80: 1055-1068.

Bower SM, McGladdery SE, Price IM (1994). Synopsis of infectious diseases and parasites of commercially exploited shellfish. *Annual Review of Fish Diseases* 4: 1-199.

Bower SM, Meyer GR, Boutillier JA (1996). Stained prawn disease (SPD) of *Pandalus platyceros* in British Columbia, Canada, caused by a rickettsial infection. *Diseases of Aquatic Organisms* 24: 41-54.

Bowser PR, Rosemark R (1981). Mortalities of cultured lobsters, *Homarus*, associated with a molt death syndrome. *Aquaculture* 23: 11-18.

Boyko CB, Williams JD (2020). A new species of *Parthenopea* Kossmann, 1874 (Cirripedia: Rhizocephala: Parthenopeidae) from Florida: the first record of a rhizocephalan from a lobster (Decapoda: Nephropoidea). *Proceedings of the Biological Society of Washington* 133: 1-6.

Breed GM, Olson RE (1977). Biology of the microsporidian parasite *Pleistophora crangoni* n. sp. in three species of crangonid sand shrimps. *Journal of Invertebrate Pathology* 30: 387-405.

Brock JA, Lightner DV (1990). *Diseases of Crustacea. Diseases caused by microorganisms*. In: O. Kinne (ed.). Diseases of Marine Animals. Volume III: Introduction, Cephalopoda, Annelida, Crustacea, Chaetognatha, Echinodermata, Urochordata. Biologische Anstalt Helgoland, Hamburg, p. 328-334.

Brock JA, Nakagawa LK, Hayashi T, Teruya S, Van Campen H (1986). Hepatopancreatic rickettsial infection of the penaeid shrimp, *Penaeus marginatus* (Randall), from Hawaii. *Journal of Fish Diseases* 9: 73-77.

Buchan KA, Martin-Robichaud DJ, Benfey TJ (2005). Measurement of dissolved ozone in sea water: A comparison of methods. Aquacultural Engineering 33: 225-231.

Burge CA, Friedman CS, Getchell R, House M, Lafferty KD, Mydlarz LD, Prager KC, Sutherland KP, Renault T, Kiryu I, Vega-Thurber R (2016). Complementary approaches to diagnosing marine diseases: a union of the modern and the classic. *Philosophical Transactions of the Royal Society B* 371: 20150207.

Burreson EM, Ford SE (2004). A review of recent information on the Haplosporidia, with special reference to *Haplosporidium nelsoni* (MSX disease). *Aquatic Living Resources* 17: 499–517.

Burreson EM, Stokes NA (2006). Chapter 5.2.2. Haplosporidiosis of oysters, In: Fish Health Section Blue Book, 2014 Edition, Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens. Section 1, Diagnostic Procedures for Finfish and Shellfish Pathogens. Chapter 5 Diseases of Molluscan Shellfish. American Fisheries Society's Fish Health Section. https://virtuallearn.wpengine.com/fhs/wp-content/uploads/sites/30/2017/08/5.2.2-Haplosporid-2014.pdf

Burreson EM, Stokes NA, Friedman CS (2000). Increased virulence in an introduced pathogen: *Haplosporidian nelsoni* (MSX) in the Eastern oyster *Crassostrea virginica*. *Journal of Aquatic Animal Health* 12: 1-8.

Butler MJ, Behringer DC (2021). Behavioral immunity and social distancing in the wild: The same as in humans ? *Bioscience* 71: 571–580.

Butler MJ, Behringer DC, Shields JD (2008). Transmission of *Panulirus argus* virus 1 (PaV1) and its effect on the survival of juvenile Caribbean spiny lobster. *Diseases of Aquatic Organisms* 79: 173–182.

Butler MJ, Behringer DC, Dolan TW, Moss J, Shields JD (2015). Behavioral immunity suppresses an epizootic in Caribbean spiny lobsters. *PLoS ONE* 10: e0126374. doi:10.1371/journal.pone.0126374

Byrne M, Cerra A, Nishigaki T, Hoshi M (1997). Infestation of the testes of the Japanese sea star *Asterias amurensis* by the ciliate *Orchitophrya stellarum*: a caution against the use of this ciliate for biological control. *Diseases of Aquatic Organisms* 28: 235–239.

Callinan R, Corsin F (2009). Audit of Diseases of Farmed Spiny Lobsters in Vietnam. Australian Centre for International Agricultural Research, ACIAR Project SMAR2008/021, 12 pgs. https://aciar.gov.au/sites/default/files/legacy/pr145-appendix_6_3-web-final.pdf

Callinan R and 12 other co-authors (2013). Determinants for WSD outbreaks in Indonesian smallholder shrimp ponds – a pilot study of locality factors, WSSV genotype distributions and pond factors. Australian Centre for International Agricultural Research (ACAIR) Report FIS/2009/035. 120 pgs.

Campbell A, Gibson R, Evans LH (1989). A new species of *Carcinomertes* (Nemertea: Carcinonemertidae) ectohabitant on *Panulirus cygnus* (Crustacea: Panuliridae) from Western Australia. *Zoological Journal of the Linnean Society* 95: 257–268.

Candia-Zulbara RI, Briones-Fourza P, Lozano-Alvarez E, Barradas-Ortiz C, Negrete-Soto F (2015). Caribbean spiny lobsters equally avoid dead and clinically PaV1-infected conspecifics. *ICES Journal of Marine Science* 72(Supplement 1): i164 –i169. doi:10.1093/icesjms/fsu249

Carnegie RB, Burreson EM (2011). Declining impact of an introduced pathogen: *Haplosporidium nelsoni* in the oyster *Crassostrea virginica* in Chesapeake Bay. *Marine Ecology Progress Series* 432: 1–15.

Carnegie RB, Hill KM, Stokes NA, Burreson EM (2014). The haplosporidian *Bonamia exitiosa* is present in Australia, but the identity of the parasite described as *Bonamia* (formerly *Mikrocytos*) *roughleyi* is uncertain. *Journal of Invertebrate Pathology* 115: 33–40.

Castro KM, Cobb JS, Gomez-Chiarri M, Tlusty M (2012). Epizootic shell disease in American lobsters *Homarus americanus* in southern New England: past, present and future. *Diseases of Aquatic Organisms* 100: 149–158.

Catanese G, Grau A, Valencia JM, Garcia-March JR, Alvarez E, Vazquez-Luis M, Deudero S, Darriba S, Carballal MJ, Villalba A (2018). *Haplosporidium pinnae* sp. nov., a haplosporidan parasite associated

with mass mortalities of the fan mussel, *Pinna nobilis*, in the Western Mediterranean Sea. *Journal of Invertebrate Pathology* 157: 9−24.

Cattaneo G (1888). Su di un infusorio ciliato, parasito del sangue del *Carcinus maenas*. *Zoologischer Anzeiger* 11: 456–459.

Cawthorn RJ, Lynn D, Despres B, MacMillan R, Maloney R, Loughlin M, Bayer R (1996). Description of *Anophryoides haemophila* n. sp. (Scuticociliatida: Orchitophryidae), a pathogen of American lobsters *Homarus americanus*. *Diseases of Aquatic Organisms* 24: 143–148.

Chaijarasphong T, Munkongwongsiri N, Stentiford GD, Aldama-Cano DJ, Thansa K, Flegel TW, Sritunyalucksana K, Itsathitphaisarn O (2021). The shrimp microsporidian *Enterocytozoon hepatopenaei* (EHP): Biology, pathology, diagnostics and control. *Journal of Invertebrate Pathology* 107458 https://doi.org/10.1016/j.jip.2020.107458

Chang PS, Chen HC, Wang YC (1998a). Detection of white spot syndrome associated baculovirus in experimentally infected wild shrimp, crabs and lobsters by *in situ* hybridization. *Aquaculture* 164: 233–242.

Chang PS, Chen LJ, Wang YC (1998b). The effect of ultraviolet irradiation, heat, pH, ozone, salinity and chemical disinfectants on the infectivity of white spot syndrome baculovirus. *Aquaculture* 166: 1-17.

Chatton E, Poisson R (1931). Sur l'existence, dans le sang des crabs, de peridiniens parasites: *Hematodinium perezi* n.g., n.sp. (Syndinidae). *CR Sceances Soc Biol Paris* 105: 553–557.

Chevrefils G, Ing B, Caron E, Wright H, Sakamoto G (2006). UV dose required to achieve incremental log inactivation of bacteria, protozoa and viruses . *IUVA News* 8: 38-45.

Chou HY, Huang CY, Wang CH, Chiang HC, Lo CF (1995). Pathogenicity of a baculovirus infection causing white spot syndrome in cultured penaeid shrimp in Taiwan. *Diseases of Aquatic Organisms* 23: 165- 173.

Clark AS (2017). Pathogen-host and environmental dynamics: A case study of *Panulirus argus* virus 1 in Caribbean spiny lobsters. PhD Dissertation. University of Florida, 2017. 112 pgs. https://ufdc.ufl.edu/UFE0051455/00001

Clark AS, Behringer DC, Moss Small J, Waltzek TB (2018). Partial validation of a TaqMan real-time quantitative PCR assay for the detection of *Panulirus argus* virus 1. *Diseases of Aquatic Organisms* 129: 193-198.

Cobcroft J, Bell,R, Fitzgerald J, Diedrich A, Jerry D. (2020). Northern Australia aquaculture situational analysis. CRC NA. 29 May 2020. 102pgs https://www.crcna.com.au/resources/publications/northern-australia-aquaculture-situational-analysis

Couch JA (1978). Diseases, parasites, and toxic responses of commercial penaeid shrimps of the Gulf of Mexico and South Atlantic coasts of North America. *Fisheries Bulletin* 76: 1–44.

Couch JA, Courtney L (1977). Interaction of chemical pollutants and virus in a crustacean: A novel bioassay system. *Annals N. Y. Academy Science* 298: 497-504.

Coutts ADM, Dodgshun TJ (2007). The nature and extent of organisms in vessel sea-chests: A protected mechanism for marine bioinvasions. *Marine Pollution Bulletin* 54: 875–886.

Crosbie PB, Munday BL (1999). Environmental factors and chemical agents affecting the growth of the pathogenic marine ciliate *Uronema nigricans*. *Diseases of Aquatic Organisms* 36: 213-219.

DAF QLD (2017). White Spot Disease detected in southern QLD. https://www.daf.qld.gov.au/business-priorities/biosecurity/animal-biosecurity-welfare/animal-health-pests-diseases/a-z-list-of-significant-animal-pests-and-diseases/white-spot-disease

DAFF (2003). Importation of pilchards (*Sardinops sagax*) for direct introduction into natural waters. Biosecurity policy review of viral haemorrhagic septicaemia virus (VHSV). Draft Report June 2003.

DAFF (2011). *Import Risk Analysis Handbook 2011.* Australian Government Department of Agriculture, Fisheries and Forestry, Canberra

Davies CE, Briones-Fourza P, Barradas-Ortiz C, Negrete-Soto F, Moo-Cocom G, Lozano-Alvarez E (2020a). Do ecological characteristics drive the prevalence of *Panulirus argus* virus 1 (PaV1) in juvenile Caribbean spiny lobsters in a tropical reef lagoon? *PLOS One* 15(2): e0229827. https://doi.org/10.1371/journal.pone.0229827

Davies CE, Bass D, Ward GM, Batista FM, Malkin SH, Thomas JE, *et al.* (2020b). Diagnosis and prevalence of two new species of haplosporidians infecting shore crabs *Carcinus maenas*: *Haplosporidium carcini* n. sp., and *H. cranc* n. sp. *Parasitology*. 147: 1229-1237.

DAWE (Department of Agriculture, Water and the Environment) (2020). Aquatic Animal Diseases Significant to Australia: Identification Field Guide, 5th Edition, Australian Government Department of Agriculture, Water and the Environment, Canberra. CC BY 4.0, 341 pgs. https://www.agriculture.gov.au/sites/default/files/documents/field-guide-5th-edition.pdf

De La Pena LD, Lavilla-Pitogo CR, Villar CB, Paner MG, Sombito CD, Capulos GC (2007). Prevalence of white spot syndrome virus (WSSV) in wild shrimp *Penaeus monodon* in the Philippines. *Diseases of Aquatic Organisms* 77: 175-179.

Dennis DM, Munday BL (1994). Microsporidiosis of palinurid lobsters from Australian waters. *Bulletin of the European Association of Fish Pathologists* 14: 16-18.

Desrina, Verreth JAJ, Prayitno SB, Rombout JH, Vlak JM, Verdegem MCJ (2013). Replication of white spot syndrome virus (WSSV) in the polychaete *Dendronereis* spp. *Journal of Invertebrate Pathology* 114: 7-10.

Diggles BK (1999). Diseases of spiny lobsters in New Zealand. Proceedings, International Symposium on Lobster Health Management, Adelaide, September 1999, pp. 18–34.

Diggles BK (2000). Diseases of spiny lobsters in New Zealand. *Journal of Shellfish Research* 19: 673.

Diggles BK (2001). A mycosis of juvenile spiny rock lobster *Jasus edwardsii* (Hutton, 1875) caused by *Haliphthoros* sp., and possible methods of chemical control. *Journal of Fish Diseases* 24: 99–110.

Diggles BK (2008). CASE REPORT: Vietnam lobsters. DigsFish Services Pathology Report DF08/05 prepared for Two Fathom Ltd, 13 May 2008.

Diggles BK (2011). Risk Analysis. Aquatic animal diseases associated with domestic bait translocation. Final report prepared for the Australian Government Department of Agriculture, Fisheries and Forestry, Canberra, FRDC Project No. 2009/072. 296 pgs. http://frdc.com.au/research/Final_Reports/2009-072-DLD.pdf

Diggles BK (2017a). Field observations and assessment of the response to an outbreak of White Spot Disease (WSD) in Black Tiger Prawns (*Penaeus monodon*) farmed on the Logan River in November 2016. FRDC Project No: 2016-064. 112 pgs.

Diggles BK (2017b). Northern Australia Biosecurity Initiative – Marine Pest and Diseases Risk Assessment. DigsFish Services Report No. DF17-02, 30 May 2017, prepared for The Northern Australia Biosecurity Initiative, Department of Agriculture and Water Resources. 194 pgs.

Diggles BK (2020a). White spot disease in Australian crustaceans. *Animal Health Surveillance Quarterly January to March 2020*, Vol 25(1): 17-21. . http://www.sciquest.org.nz/elibrary/download/165065/White_spot_disease_in_Australian_crustaceans.pdf?%22

Diggles BK (2020b). Risk Analysis - Biosecurity risks related to recycling of mollusc shell waste for shellfish reef restoration. DigsFish Services Report: DF 20-03b, for FRDC Project 2019-005, 6 November 2020, 270 pgs. http://digsfish.com/ShellrecyclingRAFINAL.pdf

Diggles BK (2020c). Survey for WSSV vectors in the Moreton Bay White Spot Biosecurity Area. DigsFish Services Report DF20-04 for Fisheries Research and Development Corporation, 15 December 2020, 54 pgs. http://digsfish.com/DigsFishreportWSSVvectors.pdf

Diggles BK, Handlinger J (2003). Disease conditions of larval and juvenile spiny lobsters. Chapter 7, pgs. 68-85, in: Evans LH (2003). Rock Lobster Autopsy Manual. FRDC Project No. 1999/202, 93 pgs.

Diggles BK, Arthur JR (2010). Pathogen risk analysis for aquatic animals: experiences from nine case studies , pp. 271-290. *In* Bondad-Reantaso, M.G., Jones, J.B., Corsin, F. and Aoki, T. (eds.). Diseases in Asian Aquaculture VII. Fish Health Section, Asian Fisheries Society, Selangor, Malaysia. 385 pgs.

Diggles BK, Moss GA, Carson J, Anderson C (2000). Luminous vibriosis in rock lobster *Jasus verreauxi* (Decapoda: Panuliridae) phyllosoma larvae caused by infection with *Vibrio harveyi*. *Diseases of Aquatic Organisms* 43: 127–137.

Diggles BK, Hine PM, Handley S, Boustead NC (2002a). A handbook of diseases of importance to aquaculture in New Zealand. National Institute of Water and Atmospheric Research, NIWA Science and Technology Series 49, Wellington.

Diggles BK, Nichol J, Hine PM, Wakefield S, Cochennec-Laureau N, Roberts RD, Friedman CS (2002b). Pathology of cultured paua (*Haliotis iris* Martyn, 1784) infected by a novel haplosporidian parasite, with some observations on the course of disease. *Diseases of Aquatic Organisms* 50: 219-231.

Diggles BK, Macbeth WG, Barnes L, Dyrting K (2013). Detection of a new rickettsia-like organism in wild-caught sand crabs (*Portunus pelagicus*) from Darwin and Bynoe Harbours. 2nd FRDC Australasian Aquatic Animal Health Scientific Conference, Pullman Reef Hotel, 8-12 July 2013, pge 21.

Diggles BK, Chong RSM, Hawkins KA, Underwood DJ, Genge B, Driver L, Kistler C (2020). Investigation into white spots in the carapace of a moribund mud crab (*Scylla serrata*) from a white spot syndrome virus (WSSV) positive zone in Moreton Bay, Australia. *Australian Veterinary Journal* 98: 550-554.

Diggles BK, Bass D, Bateman KS, Chong R, Daumich C, Hawkins KA, Hazelgrove R, Kerr R, Moody NC, Ross S, Stentiford GD (submitted). *Haplosporidium acetes* n.sp. infecting the hepatopancreas of jelly prawns *Acetes sibogae australis* from Moreton Bay, Australia. *Journal of Invertebrate Pathology*

Di Giorgio G, Gilardoni C, Ituarte C (2014). Pathology of *Haplosporidium patagon* affecting siphonariid gastropods in Patagonia. *Diseases of Aquatic Organisms* 112: 59-67.

Ding Z, Meng Q, Liu H, Yuan S, Zhang F, Sun M, Zhao Y, Shen M, Zhou1 G, Pan J, Xue H, Wang W (2016). First case of hepatopancreatic necrosis disease in pond-reared Chinese mitten crab, *Eriocheir sinensis*, associated with microsporidian. *Journal of Fish Diseases* 39: 1043-1051.

Dove ADM, LoBue C, Bowser P, Powell M (2004). Excretory calcinosis: a new fatal disease of wild American lobsters *Homarus americanus*. *Diseases of Aquatic Organisms* 58: 215-221.

Durand SV, Tang KFJ, Lightner DV (2000). Frozen commodity shrimp: potential avenue for introduction of white spot syndrome virus and yellowhead virus. *Journal of Aquatic Animal Health* 12: 128-135.

Dykova I, Lom J, Fajer E (1988). A new haplosporean infecting the hepatopancreas in the penaeid shrimp, *Penaeus vannamei*. *Journal of Fish Diseases* 11: 15-22.

East IJ, Black PF, McColl KA, Hodgson R, Bernoth EM (2004). Survey for the presence of white spot syndrome virus in Australian crustaceans. *Australian Veterinary Journal* 82: 236-240.

East IJ, Black PF, Findlay VL, Bernoth EM (2005). A national survey to verify freedom from white spot syndrome virus and yellowhead virus in Australian crustaceans. In Diseases in Asian Aquaculture V (ed. By P.Walker, R. Lester & M.G. Bondad-Reantaso), pgs.15-26. Fish Health Section, Asian Fisheries Society, Manila, Philippines.

Eaton WD, Love DC, Botelho C, Meyers TR, Imamura K, Koeneman T (1991). Preliminary results on the seasonality and life cycle of the parasitic dinoflagellate causing Bitter Crab Disease in Alaskan Tanner crabs (*Chionoecetes bairdi*). *Journal of Invertebrate Pathology* 57: 426–434.

Eddy F, Powell A, Gregory S, Nunan LM, Lightner DV, Dyson PJ, Rowley AF, Shields RJ (2007). A novel bacterial disease of the European shore crab, *Carcinus maenas* – molecular pathology and epidemiology. *Microbiology* 153, 2839–2849.

Edgerton BF (2004). Susceptibility of the Australian freshwater crayfish *Cherax destructor albidus* to white spot syndrome virus (WSSV). *Diseases of Aquatic Organisms* 59: 187-193.

Edgerton BF, Prior HC (1999). Description of a hepatopancreatic rickettsia-like organism in the redclaw crayfish *Cherax quadricarinatus Diseases of Aquatic Organisms* 36: 77-80.

Edgerton BF, Owens L, Harris L, Thomas A, Wingfield M (1995). A health survey of farmed redclaw crayfish, *Cherax quadricarinatus* (von Martens), in tropical Australia. *Freshwater Crayfish* 10: 322-338.

Edgerton BF, O'Donoghue P, Wingfield M, Owens L (1996). Systemic infection of freshwater crayfish *Cherax quadricarinatus* by hymenostome ciliates of the *Tetrahymena pyriformis* complex. *Diseases of Aquatic Organisms* 27: 123–129.

Edgerton BF, Evans LH, Stephens FJ, Overstreet RM (2002). Synopsis of freshwater crayfish diseases and commensal organisms. *Aquaculture* 206: 57-135.

Elliman JR, Owens L (2020). Confirmation that candidatus *Coxiella cheraxi* from redclaw crayfish (*Cherax quadricarinatus*) is a close relative of *Coxiella burnetii*, the agent of Q-fever. *Letters in Applied Microbiology* 71:320-326.

Elston RA, Cheney D, Frelier P, Lynn D (1999). Invasive orchitophryid ciliate infections in juvenile Pacific and Kumomoto oysters, *Crassostrea gigas* and *Crassostrea sikamea*. *Aquaculture* 174: 1–14.

Esparza-Leal HM, Escobedo-Bonilla CM, Casillas-Hernández R, Álvarez-Ruíz P, Portillo-Clark G, Valerio-García RC, Hernández-López J, Méndez-Lozano J, Vibanco-Pérez N, Magallón-Barajas FJ (2009). Detection of white spot syndrome virus in filtered shrimp-farm water fractions and experimental evaluation of its infectivity in *Penaeus* (*Litopenaeus*) *vannamei*. *Aquaculture* 292: 16–22.

Evans LH (2003). A review of lobster diseases, their investigation and predisposing factors. Fisheries Research and Development Corporation Report FRDC Project No. 1999/202. In: Evans LH (2003). Rock Lobster Autopsy Manual. FRDC Project No. 1999/202, 93 pgs.

Field RH, Appleton PL (1995). A *Hematodinium*-like dinoflagellate infection of the Norway lobster *Nephrops norvegicus*: observations on pathology and progression of infection. *Diseaes of Aquatic Organisms* 22: 115-128.

Field RH, Chapman CJ, Taylor AC, Neil DM, Vickerman K (1992). Infection of the Norway lobster *Nephrops norvegicus* by a *Hematodinium*-like species of dinoflagellate on the west coast of Scotland. *Diseaes of Aquatic Organisms* 13: 1–15.

Fernandez-Boo S, Provot C, Lecadet C, Stavrakakis C, Papin M, Chollet B, Auvray JF, Arzul I (2021). Inactivation of marine bivalve parasites using UV-C irradiation: Examples of *Perkinsus olseni* and *Bonamia ostreae*. *Aquaculture Reports* 21: 100859.

Fisher WS, Nilson EH, Shleser RA (1975). Effect of the fungus *Haliphthoros milfordensis* on the juvenile stages of the American lobster *Homarus americanus*. *Journal of Invertebrate Pathology* 26: 41–45.

Fisher WS, Nilson EH, Follett LF, Shleser RA (1976). Hatching and rearing lobster larvae (*Homarus americanus*) in a disease situation. *Aquaculture* 7: 75–80.

Fisher WS, Nilson EH, Steenbergen JF, Lightner DV (1978). Microbial diseases of cultured lobsters: a review. *Aquaculture* 14: 115–140.

Flegel TW (2006). Detection of major penaeid shrimp viruses in Asia, a historical perspective with emphasis on Thailand. *Aquaculture* 258: 1-33.

Flegel TW (2009). Review of disease transmission risks from prawn products exported for human consumption. *Aquaculture* 290: 179-326.

Flegel TW, Boonyaratpalin S, Fegan DF, Guerin M, Sriurairatana S (1992). High mortality of black tiger prawns from cotton shrimp disease in Thailand. In: *Diseases in Asian Aquaculture I*: 181-197.

Ford SE, Bushek D (2012). Development of resistance to an introduced marine pathogen by a native host. *Journal of Marine Research*, 70: 205–223.

Ford SE, Xu Z, Debrosse G (2001). Use of particle filtration and UV irradiation to prevent infection by *Haplosporidium nelsoni* (MSX) and *Perkinsus marinus* (Dermo) in hatchery reared larval and juvenile oysters. *Aquaculture* 194: 37-49.

Ford SE, Allam B, Xu Z (2009). Using bivalves as particle collectors with PCR detection to investigate the environmental distribution of *Haplosporidium nelsoni*. *Diseases of Aquatic Organisms* 83: 159-168.

Ford SE, Stokes NA, Alcox KA, Flores Kraus BS, Barber RD, Carnegie RB, Burreson EM (2018). Investigating the life cycle of *Haplosporidium nelsoni* (MSX): A review. *Journal of Shellfish Research* 37: 679-693.

Freeman DJ, MacDiarmid AB (2009). Healthier lobsters in a marine reserve: effects of fishing on disease incidence in the spiny lobster, *Jasus edwardsii*. *Marine and Freshwater Research* 60: 140–145.

Friedman CS (1996). Haplosporidian infections of the Pacific oyster, *Crassostrea gigas* (Thunberg), in California and Japan. *Journal of Shellfish Research* 15: 597-600.

Frischer ME, Lee RF, Sheppard MA, Mauer A and others (2006). Evidence for a free-living life stage of the blue crab parasitic dinoflagellate, *Hematodinium* sp. *Harmful Algae* 5: 548–557.

Future Fisheries Veterinary Service (2017). Assessing compliance and efficacy of import conditions for uncooked prawn in relation to White Spot Syndrome Virus (WSSV). FRDC Project 2016-066 Report to Australian Prawn farmers Association. 103 pgs.

Gaughan DJ (2002). Disease-translocation across geographic boundaries must be recognised as a risk even in the absence of disease identification: the case with Australian *Sardinops*. *Reviews in Fish Biology and Fisheries* 11: 113-123.

Geddes MC, Musgrave RJ, Thomas CJ (2003). Investigation of tail fan necrosis in live-held adult southern rock lobsters. FRDC Project 2000/211.

Glenner H, Thomsen PF, Rybakov AV, Galil BS, Hoeg JT (2008). The phylogeny of rhizocephalan parasites of the genus *Heterosaccus* using molecular and larval data (Cirripedia: Rhizocephala; Sacculinidae). *Israel Journal of Ecology and Evolution* 54: 223-238.

Gornik SG, Cranenburgh A, Waller RF (2013). New host range for *Hematodinium* in Southern Australia and novel tools for sensitive detection of parasitic dinoflagellates. *PLoS ONE* 8: e82774. doi:10.1371/journal.pone.0082774.

Greenwood SJ, Despres BM, Cawthron RJ, Lavallee J, Groman DB, Desbarats A (2005). Case report: Outbreak of bumper car disease caused by *Anophryoides haemophila* in a lobster holding facility in Nova Scotia, Canada. *Journal of Aquatic Animal Health* 17: 345–352.

Gurney RH, Rybakov AV, Høeg JT, Kuris AM (2006). *Sacculina nectocarcini*, a new species of rhizocephalan (Cirripedia: Rhizocephala) parasitising the red rock crab *Nectocarcinus integrifrons* (Decapoda: Brachyura: Portunidae). *Zootaxa* 1332: 37-50.

Hall MR, Kenway M, Salmon M, Francis D, Goulden EF, Høj L (2013). Palinurid lobster larval rearing for closed-cycle hatchery production. Pages 289-328, In: Advances in Aquaculture Hatchery Technology Woodhead Publishing Series in Food Science, Technology and Nutrition.

Han JE, Tang KF, Lightner DV (2016). New management tools for EHP in penaeid shrimp. *Global Aquaculture Advocate* 15 April, 2016.

Handlinger J, Carson J, Ritar A, Crear B, Taylor DP, Johnston D (1999). Disease conditions of cultured phyllosoma and juveniles of the southern rock lobster (*Jasus edwardsii*, Decapoda; Palinuridae). Proceedings, International Symposium on Lobster Health Management, Adelaide, September 1999, pgs 75– 87.

Handlinger J, Carson J, Ritar A, Crear B (2000). A study of diseases in cultured phyllosoma larvae and juveniles of southern rock lobster (*Jasus edwardsii*). *Journal of Shellfish Research* 19: 676.

Hartikainen H, Ashford OS, Berney C, Okamura B, Feist SW, Baker-Austin C, Stentiford GD, Bass D (2014). Lineage-specific molecular probing reveals novel diversity and ecological partitioning of haplosporidians. *The ISME Journal* 8: 177–186.

Haskin HH, Ford SE (1982). *Haplosporidium nelsoni* (MSX) on Delaware Bay seed oyster beds: A host-parasite relationship along a salinity gradient. *Journal of Invertebrate Pathology* 40: 388-405.

Haskin HH, Andrews JD (1988). Uncertainties and speculations about the life cycle of the eastern oyster pathogen *Haplosporidium nelsoni* (MSX). In: Fisher WS (ed) Disease processes in marine bivalve molluscs. *American Fisheries Society Special Publication* 18: 5-22.

Hasson KW, Fan Y, Reisinger T, Venuti J, Varner PW (2006). White spot syndrome virus (WSSV) introduction into the Gulf of Mexico and Texas freshwater systems through imported frozen bait shrimp. *Diseases of Aquatic Organisms* 71: 91-100.

Herrera-Salvatierra N, Pascual-Jiménez C, Huchin-Mian JP, Lozano-Álvarez E, Montero-Muñoz J, Briones-Fourzán P, Rodríguez-Canul R (2019). Nutritional and immunological evaluation of juvenile spiny lobsters *Panulirus argus* (Latreille, 1804) (Decapoda: Achelata: Palinuridae) naturally infected with the PaV1 virus. *Journal of Crustacean Biology* 39: 162–171.

Heersink D, Paini D, Caley P, Barry S (2014). Asian Green Mussel: estimation of approach rate and probability of invasion via biofouling. Plant Biosecurity CRC - Research Services Final Report 28 pgs. https://publications.csiro.au/rpr/pub?pid=csiro:EP172373

Herbert BW (1988). Infection of *Cherax quadricarinatus* (Decapoda: Parastacidae) by the microsporidian *Thelohania* sp. (Microspora: Nosematidae). *Journal of Fish Diseases* 11: 301-308.

Herbert B, Graham P (2008). Tetrahymenosis, columnaris disease and motile aeromonad septicaemia in golden perch, *Macquaria ambiqua* (Richardson), from Australia, pp. 179-192. In: Bondad-Reantaso MG, Mohan CV, Crumlish M, Subasinghe RP (eds.). Diseases in Asian Aquaculture VI. Fish Health Section, Asian Fisheries Society, Manila, Philippines. 505 pgs.

Hine PM (2020). Haplosporidian host:parasite interactions. *Fish and Shellfish Immunology* 103: 190–199.

Hine PM, Thorne T (1998). *Haplosporidium* sp. (Haplosporidia) in hatchery-reared pearl oysters, *Pinctada maxima* (Jameson, 1901), in north Western Australia. *Journal of Invertebrate Pathology* 71: 48–52.

Hine PM, Thorne T (2000). A survey of some parasites and diseases of several species of bivalve mollusc in northern Western Australia. *Diseases of Aquatic Organisms* 40: 67-78.

Hine PM, Thorne T (2002). *Haplosporidium* sp. (Alveolata: Haplosporidia) associated with mortalities among rock oysters *Saccostrea cucullata* in north Western Australia. *Diseases of Aquatic Organisms* 51: 123–133.

Hine PM, Wakefield S, Diggles BK, Webb VL, Maas EW (2002). The ultrastructure of a haplosporidian containing Rickettsiae, associated with mortalities among cultured paua *Haliotis iris*. *Diseases of Aquatic Organisms* 49: 207-219.

Hine PM, Carnegie RB, Burreson EM, Engelsma MY (2009). Inter-relationships of haplosporidians deduced from ultrastructural studies. *Diseases of Aquatic Organisms* 83: 247-256.

Høj L, Bourne DG, Hall MR (2009). Localization, abundance and community structure of bacteria associated with *Artemia*: Effects of nauplii enrichment and antimicrobial treatment. *Aquaculture* 293: 278–285.

Holt CC, Foster R, Daniels CL, van der Giezen M, Feist SW, Stentiford GD, Bass D (2018). *Halioticida noduliformans* infection in eggs of lobster (*Homarus gammarus*) reveals its generalist parasitic strategy in marine invertebrates. *Journal of Invertebrate Pathology* 154: 109-116.

Holt CC, Stone M, Bass D, Bateman KS, van Aerle R, Daniels CL, van der Giezen M, Ross SH, Hooper C, Stentiford GD (2019). The first clawed lobster virus *Homarus gammarus* nudivirus (HgNV n. sp.) expands the diversity of the Nudiviridae. *Nature Scientific Reports* 9: 10086 . https://doi.org/10.1038/s41598-019-46008-y

Holt CC, van der Giezen M, Daniels CL, Stentiford GD, Bass D (2020). Spatial and temporal axes impact ecology of the gut microbiome in juvenile European lobster (*Homarus gammarus*). *The ISME Journal* 14: 531–543.

Huang Q, Li M, Wang F, Li C (2019). The parasitic dinoflagellate *Hematodinium perezi* infecting mudflat crabs, *Helice tientsinensis*, in polyculture system in China. *Journal of Invertebrate Pathology* 166: 107229.

Huchin-Mian JP, Rodríguez-Canul R, Arias-Bañuelos E, Simá-Álvarez R, Briones-Fourzán P, Lozano-Álvarez E (2008). Presence of *Panulirus argus* Virus 1 (PaV1) in juvenile spiny lobsters *Panulirus argus* from the Caribbean coast of Mexico. *Diseases of Aquatic Organisms* 79: 153−156.

Huchin-Mian JP, Briones-Fourzán P, Simá-Álvarez R, Cruz-Quintana Y, Antonio Pérez-Vega Y, Lozano-Álvarez E, Pascual-Jiménez C, Rodríguez-Canul R (2009). Detection of *Panulirus argus* Virus 1 (PaV1) in exported frozen tails of subadult-adult Caribbean spiny lobsters *Panulirus argus*. *Diseases of Aquatic Organisms* 86: 159−162.

Huchin-Mian JP, Small HJ, Shields JD (2017). Patterns in the natural transmission of the parasitic dinoflagellate *Hematodinium perezi* in American blue crabs, *Callinectes sapidus* from a highly endemic area. *Marine Biology* 164: 153.

Huchin-Mian JP, Small HJ, Shields JD (2018). The influence of temperature and salinity on mortality of recently recruited blue crabs, *Callinectes sapidus*, naturally infected with *Hematodinium perezi* (Dinoflagellata). *Journal of Invertebrate Pathology* 152: 8–16.

Hudson DA, Lester RJG (1994). Parasites and symbionts of wild mud crabs *Scylla serrata* (Forskal) of potential significance in aquaculture. *Aquaculture* 120: 183–199.

Hudson DA, Shields JD (1994). *Hematodinium australis* n. sp., a parasitic dinoflagellate of the sand crab *Portunus pelagicus* from Moreton Bay, Australia. *Diseases of Aquatic Organisms* 19: 109–119.

Hudson DA, Adlard RD (1994). PCR-techniques applied to *Hematodinium* spp and *Hematodinium*-like dinoflagellates in decapod crustaceans. *Diseases of Aquatic Organisms* 20: 203–206.

Hudson DA, Adlard RD (1996). Nucleotide sequence determination of the partial SSU rDNA gene and ITS1 region of *Hematodinium* cf. *perezi* and *Hematodinium*-like dinoflagellates. *Diseases of Aquatic Organisms* 24: 55–60.

Hudson DA, Hudson NB, Pyecroft SB (2001). Mortalities of *Penaeus japonicus* prawns associated with microsporidean infection. *Australian Veterinary Journal* 79: 504-505.

Hung LV, Tuan LA (2009). Lobster seacage culture in Vietnam. In: Williams K.C. (ed.) 2009. Spiny lobster aquaculture in the Asia–Pacific region. Proceedings of an international symposium held at Nha Trang, Vietnam, 9–10 December 2008. ACIAR Proceedings No. 132. Australian Centre for International Agricultural Research: Canberra. 162 pgs. https://www.cabdirect.org/cabdirect/abstract/20093336787

Hung L, Khuong D, Phuoc T, Thao M (2010). Relative efficacies of lobsters (*Panulirus ornatus* and *P. homarus*) cultured using pellet feeds and "trash" fish at Binh Ba Bay, Vietnam. *Aquaculture Asia Magazine* 15: 3–6.

Inouye KS, Miwa S, Oseko N, Nakano H, Kimura T, Momoyama K. Hiraoka M (1994). Mass mortality of cultured kuruma shrimp *Penaeus japonicus* in Japan in 1993: Electron microscopic evidence of the causative virus. *Fish Pathology* 29: 149-158.

Itoh N, Kuboyama A, Freeman MA, Katata M, Yamakawa T, Yoshinaga T (2020). A novel dimorphic microsporidian *Ameson iseebi* sp. nov. infecting muscle of the Japanese spiny lobster, *Panulirus japonicus*, in western Japan. *Journal of Invertebrate Pathology* 176: 107472.

Ituarte C, Bagnato E, Siddall ME, Cremonte F (2014). A new species of *Haplosporidium* Caullery & Mesnil, 1899 in the marine false limpet *Siphonaria lessonii* (Gastropoda: Siphonariidae) from Patagonia. *Systematic Parasitology* 88: 63−73.

Jacangelo JG, Patania NL, Rhodes Trussell R, Haas CN, Gerba C (2002). Inactivation of waterborne emerging pathogens by selected disinfectants. *American Water Works Association and US EPA*, Washington, USA, 145 pgs.

Jensen PC, Califf K, Lowe V, Hauser L, Morado JF (2010). Molecular detection of *Hematodinium* sp. in Northeast Pacific *Chionoecetes* spp. and evidence of two species in the Northern Hemisphere. *Diseases of Aquatic Organisms* 89: 155–166.

Jensen MA, Ritar AJ, Burke C, Ward LR (2011). Seawater ozonation and formalin disinfection for the larval culture of eastern rock lobster, *Jasus* (*Sagmariasus*) *verreauxi*, phyllosoma. *Aquaculture* 318: 213-222.

John DE, Haas CN, Nwachuku N, Gerba CP (2005). Chlorine and ozone disinfection of *Encephalitozoon intestinalis* spores. *Water Research* 39: 2369–2375.

Jones CM (2015). Spiny lobster aquaculture development in Indonesia, Vietnam and Australia. Proceedings of the International Lobster Aquaculture Symposium held in Lombok, Indonesia, 22–25 April 2014. ACIAR Proceedings No. 145. Australian Centre for International Agricultural Research: Canberra. 285 pgs.

Jones DS (2003). The biogeography of Western Australian shallow-water barnacles. In: Wells FE, Walker DI, Jones DS (Eds.), *The marine flora and fauna of Dampier, Western Australia*. Western Australian Museum, Perth, pp. 479–496.

Jones DS (2004). Barnacles (Cirripedia: Thoracica) of the Dampier Archipelago, Western Australia. *Records of the Western Australian Museum Supplement* 66: 121–157.

Jones JB (2012). Transboundary movement of shrimp viruses in crustaceans and their products: A special risk ? *Journal of Invertebrate Pathology* 110: 196–200.

Jones JB, Lawrence CS (2001) Diseases of yabbies (*Cherax albidus*) in Western Australia. *Aquaculture* 194: 221-232.

Jones JB, Creeper J (2006). Diseases of pearl oysters and other molluscs: A Western Australian perspective. *Journal of Shellfish Research* 25: 233-238.

Jones JB, Stephens F (2006). Aquatic Animal Health Subprogram: development of a national translocation policy using abalone and prawns as templates for other aquatic species. FRDC Project 2004/080. 86 pgs.

Kamaishi T, Yoshinaga T (2002). Detection of *Haplosporidium nelsoni* in Pacific oyster *Crassostrea gigas* in Japan. *Fish Pathology* 37: 193-195.

Kanchanaphuam P, Wongteerasupaya C, Sitidilokratana N, Boonsaeng V, Panyim S, Tassanakajon A, Withyachumnarnkul B, Flegel TW (1998). Experimental transmission of white spot syndrome virus (WSSV) from crabs to shrimp *Penaeus monodon*. *Diseases of Aquatic Organisms* 34: 1-7.

Kantar Public (2019). White spot disease market research. Report prepared for Biosecurity QLD, May 2019.

Kasai H, Osawa S, Kobayashi T, Yoshimizu M (2002a). Prevention of scuticociliatosis in Japanese flounder by treatment of water supply with a high quality UV lamp. *Fish Pathology* 37: 199-200.

Kasai H, Yoshimizu M, Ezura Y (2002b). Disinfection of water for aquaculture. *Fisheries Science* 68 (Suppl 1): 821-824.

Kawato S, Shitara A, Wang Y, Nozaki R, Kondo H, Hirono I (2019). Crustacean genome exploration reveals the evolutionary origin of white spot syndrome virus. *Journal of Virology* 93: e01144-18.

Ke F, Gui L, Li T, Li FH, Zhao X, Chen ZY, Lei CK, Zhang QY (2021). The features of an emerging whispovirus isolate from freshwater crayfish. *Aquaculture Reports* 20: 100728.

Keeling PJ (2014). Phylogenetic place of Microsporidia in the tree of eukaryotes. In: *Microsporidia: Pathogens of Opportunity*, 1st Ed. Becnel JJ, Weiss LM (eds). Wiley Blackwell, pgs 195–220.

Kent ML, Feist SW, Harper C, Hoogstraten-Miller S, Law M, Sánchez-Morgado JM, Tanguay RL, Sanders GE, Spitsbergen JM, Whipps CM (2009). Recommendations for control of pathogens and infectious diseases in fish research facilities. *Comparative Biochemistry and Physiology*, Part C 149: 240–248.

Ketterer PJ, Taylor DJ, Prior HC (1992). Systematic rickettsia-like infection in farmed freshwater crayfish, *Cherax quadricarinatus*. In: Shariff M, Subasinghe RP, Arthur JR (eds) Diseases in Asian aquaculture 1. Fish Health Section, Asian Fisheries Society, Manila, Philippines, pgs 173-179.

Kesavan K, Mani R, Toshiaki I, Sudhakaran R (2016). Quick report on prevalence of shrimp microsporidian parasite *Enterocytozoon hepatopenaei* in India. *Aquaculture Research* 48: 3980- 3984.

Kewagama Research (2007). *National survey of bait and berley use by recreational fishers: a follow-up survey focussing on prawns/shrimp*. Report to: Biosecurity Australia, AFFA.

Kitancharoen N, Hatai K (1995). A marine oomycete *Atkinsiella panulirata* sp. nov. from philozoma of spiny lobster, *Panulirus japonicus*. *Mycoscience* 36: 97–104.

Kittaka J (1997). Culture of larval spiny lobsters: a review of work done in northern Japan. *Marine and Freshwater Research* 48: 923–930.

Kiryu Y, Behringer DC, Landsberg JH, Petty BD (2009). Microsporidiosis in the Caribbean spiny lobster *Panulirus argus* from southeast Florida, USA. *Diseases of Aquatic Organisms* 84: 237–242.

Kough AS, Paris CB, Behringer DC, Butler MJ (2015). Modelling the spread and connectivity of waterborne marine pathogens: the case of PaV1 in the Caribbean. *ICES Journal of Marine Science* 72 (Supplement 1): i139–i146. doi:10.1093/icesjms/fsu209.

Krol RM, Hawkins WE, Overstreet RM (1991). Rickettsial and mollicute infections in hepatopancreatic cells of cultured pacific white shrimp (*Penaeus vannamei*). *Journal of Invertebrate Pathology* 57: 362-370.

Langdon JS (1990). Disease risks of fish introductions and translocations. *Bureau of Rural Resources Proceedings* 8: 98–107.

Langdon JS (1991). Description of *Vavraia parastacida* sp. nov. (Microspora: Pleistophoridae) from marron, *Cherax tenuimanus* Smith (Decapoda: Parastacidae). *Journal of Fish Diseases* 14: 619–629.

Langdon JS, Thorne T (1992). Experimental transmission per-os of microsporidosis due to *Vavraia parastacida* in the marron, *Cherax tenuimanus* (Smith) and yabby *Cherax albidus* Clark. *Journal of Fish Diseases* 15: 315-322.

Larsson JI (1987). On *Haplosporidium gammari*, a parasite of the amphipod *Rivulogammarus pulex*, and its relationships with the phylum Ascetospora. *Journal of Invertebrate Pathology* 49: 159-169.

Lavallée J, Hammell KL, Spangler ES, Cawthorn RJ (2001). Estimated prevalence of *Aerococcus viridans* and *Anophryoides haemophila* in American lobsters *Homarus americanus* freshly captured in the waters of Prince Edward Island, Canada. *Diseases of Aquatic Organisms* 46: 231-236.

Li X, Fayer R (2006). Infectivity of microsporidian spores exposed to temperature extremes and chemical disinfectants. *Journal of Eukaryotic Microbiology* 53(S1): S77–S79.

Li CW, Shields JD, Ratzlaff RE, Butler MJ (2008a). Pathology and hematology of the Caribbean spiny lobster experimentally infected with *Panulirus argus* virus 1 (PaV1). *Virus Research* 132: 104–113.

Li YY, Xia XA, Wu QY, Liu WH, Lin YS (2008b). Infection with *Hematodinium* sp. in mud crabs *Scylla serrata* cultured in low salinity water in southern China. *Diseases of Aquatic Organisms* 82: 145-150.

Li CW, Wheeler KN, Shields JD (2011a). Lack of transmission of *Hematodinium* sp. in the blue crab *Callinectes sapidus* through cannibalism. *Diseases of Aquatic Organisms* 96: 249–258.

Li CW, Miller TL, Small HJ, Shields JD (2011b). *In vitro* culture and developmental cycle of the parasitic dinoflagellate *Hematodinium* sp. from the blue crab *Callinectes sapidus*. *Parasitology* 138: 1924–1934.

Li CW, Song SQ, Liu Y, Chen TT (2013). *Hematodinium* infections in cultured Chinese swimming crab, *Portunus trituberculatus*, in northern China. *Aquaculture* 396–399: 59–65.

Li CW, Li M, Huang Q (2021). The parasitic dinoflagellate *Hematodinium* infects marine crustaceans. *Marine Life Science & Technology* 3: 313–325.

Lightner DV (1996a). Epizootiology, distribution and the impact on international trade of two penaeid shrimp viruses in the Americas. *Revue Scientifique et Technique Office International des Epizooties* 15: 579- 601.

Lightner DV (1996b). *A Handbook of Shrimp Pathology and Diagnostic Procedures for Diseases of Cultured Penaeid Shrimp*. World Aquaculture Society, Baton Rouge, Louisiana, USA.

Lightner DV (2003). The penaeid shrimp viral pandemics due to IHHNV, WSSV, TSV, & YHV: Current status in the Americas. Presented at U. California, Davis, CA on 11/17/2003. 32nd UJNR (US-Japan Cooperative Program in Natural Resources) Aquaculture Panel Meeting, November 16-22, 2003.

Lightner DV (2005). Biosecurity in shrimp farming: Pathogen exclusion through use of SPF stock and routine surveillance. *Journal of the World Aquaculture Society* 36: 229-248.

Lightner DV, Redman RM (1994). An epizootic of necrotizing hepatopancreatitis in cultured penaeid shrimp (Crustacea: Decapoda) in northwestern Peru. *Aquaculture* 122: 9-18.

Lightner DV, Redman RM, Poulos BT, Nunan LM, Mari JL, Hasson KW (1997). Risk of spread of penaeid shrimp viruses in the Americas by the international movement of live and frozen shrimp. *Revue Scientifique et Technique Office International des Epizooties* 16: 146–160.

Lightner DV, Pantoja CP, Redman RM, Poulos BT, Nguyen HD, Do TH, Nguyen TC (2008). Collaboration on milky disease of net-pen-reared spiny lobsters in Vietnam. *OIE Bulletin* 2: 46-47. https://www.oie.int/app/uploads/2021/03/bull-2008-2-eng.pdf

Liltved H, Cripps SJ (1999). Removal of particle-associated bacteria by prefiltration and ultraviolet irradiation. *Aquaculture Research* 30: 445–450.

Liltved H, Vogelsang C, Modahl I, Dannevig BH (2006). High resistance of fish pathogenic viruses to UV irradiation and ozonated seawater. *Aquacultural Engineering* 34: 72–82.

Lin Q, Lim JY, Xue K, Yew PY, Owh C, Chee PL, Loh XJ (2020). Sanitizing agents for virus inactivation and disinfection. VIEW 2020 e16, https://doi.org/10.1002/viw2.16

Liu X, Lei Y, Ren Z, Zhou S, Qian D, Yu Y, Yin F, Wang C (2020). Isolation, characterization and virulence of *Mesanophrys* sp. (Ciliophora: Orchitophryidae) in farmed swimming crab (*Portunus trituberculatus*) in eastern China. *Journal of Fish Diseases* 43: 1419–1429.

Lo CF, Kou GH (1998). Virus-associated white spot syndrome of shrimp in Taiwan: A review. *Fish Pathology* 33: 365-371.

Lo CF, Ho CH, Peng SE, Chen CH, Hsu HC, Chiu YL, Chang CF, Liu KF, Su MS, Wang CH, Kou GH (1996). White spot syndrome baculovirus (WSBV) detected in cultured and captured shrimp, crabs and other arthropods *Diseases of Aquatic Organisms* 27: 215-225.

Lo CF, Ho CH, Chen CH, Liu KF, Chiu YL, Yeh PY, Peng SE, Hsu HC, Liu HC, Chang CF, Su MS, Wang CH, Kou GH (1997). Detection and tissue tropism of white spot syndrome baculovirus (WSBV) in captured brooders of *Penaeus monodon* with a special emphasis on reproductive organs. *Diseases of Aquatic Organisms* 30: 53–72.

Lom J, Dykova I (1992). *Protozoan Parasites of Fishes*. Developments in Aquaculture and Fisheries Science 26. Elsevier. 315 pgs.

Lozano-Álvarez E, Briones-Fourzán P, Ramírez-Estévez A, Placencia-Sánchez D, Huchin-Mian JP, Rodríguez-Canul R (2008). Prevalence of *Panulirus argus* Virus 1 (PaV1) and habitation patterns of healthy and diseased Caribbean spiny lobsters in shelter-limited habitats. *Diseases of Aquatic Organisms* 80: 95−104.

Lozano-Álvarez E, Briones-Fourzán P, Huchin-Mian JP, Segura-García I, Ek-Huchim JP, Améndola-Pimenta M, Rodríguez-Canul R (2015). *Panulirus argus* virus 1 detected in oceanic postlarvae of Caribbean spiny lobster: implications for disease dispersal. *Diseases of Aquatic Organisms* 117: 165-170.

Macias-Rodríguez NA, Mañón-Ríos N, Romero-Romero JL, Camacho-Beltrán E, Magallanes-Tapia MA, Leyva-López NE, Hernández-López J, Magallón-Barajas FJ, Perez-Enriquez R, Sánchez-González S, Méndez-Lozano J (2014). Prevalence of viral pathogens WSSV and IHHNV in wild organisms at the Pacific Coast of Mexico. *Journal of Invertebrate Pathology* 116: 8–12.

Maddox JV, Solter LF (1996). Long-term storage of infective microsporidian spores in liquid nitrogen. *Journal of Eukaryotic Microbiology* 43: 221–225.

Maeda M, Itami T, Furumoto A, Henning O, Imamura T, Kondo M, Hirono I, Takashi A, Takahashi Y (1998a). Detection of penaeid rod-shaped DNAvirus (PRDV) in wild-caught shrimp and other crustaceans. *Fish Pathology* 33: 373–380.

Maeda M, Kasornchandra J, Itami T, Suzuki N, Henning O, Kondo M, Albaladejo JD, Takahashi Y (1998b). Effect of various treatments on white spot syndrome virus (WSSV) from *Penaeus japonicus* (Japan) and *P. monodon* (Thailand). *Fish Pathology* 33: 381–387.

Marchand J, Sprague V (1979). Ultrastructure de *Minchinia cadomensis* sp. n. (Haplosporida) Parasite du Décapode *Rhithropanopeus harrisii* tridentatus Maitland dans le Canal de Caen à la Mer (Calvados, France). *The Journal of Protozoology* 26: 179-185.

Marchese G, Fitzgibbon QP, Trotter AJ, Carter CG, Jones CM, Smith GG (2019). The influence of flesh ingredients format and krill meal on growth and feeding behaviour of juvenile tropical spiny lobster *Panulirus ornatus*. *Aquaculture* 499: 128-139.

Mathis A, Weber R, Deplazes P (2005). Zoonotic potential of the Microsporidia. *Clinical Microbiology Reviews* 18: 423–445.

Mayo MA (2005). Changes to virus taxonomy 2004. *Archives of Virology* 150: 189–198.

McColl KA, Slater J, Jeyasekaran G, Hyatt AD, Crane M (2004). Detection of white spot syndrome virus and yellowhead virus in prawns imported in Australia. *Australian Veterinary Journal* 82: 69-74.

McAleer R, Baxter M (1983). Black shell disease of the western rock lobster caused by *Fusarium solani*. In: Proc. 8th Cong. Internat. Soc. Human Anim. Mycol. Massey University, Palmerston North, New Zealand, 1982, pp. 378–382.

Mendoza-Cano F, Sanchez-Paz A, Teran-Dıaz B, Galvan-Alvarez D, Encinas-Garc T, Enrıquez-Espinoza T, Hernandez-Lopez G (2014). The endemic copepod *Calanus pacificus californicus* as a potential vector of White Spot Syndrome Virus. *Journal of Aquatic Animal Health* 26: 113–117.

Meng XH, Jang IK, Seo HC, Cho YR (2009). White spot syndrome virus quantification in blue crab *Portunus trituberculatus* hatchery-produced larvae and wild populations by TaqMan real-time PCR, with an emphasis on the relationship between viral infection and crab health. *Aquaculture* 291: 18–22.

Messerman NA, Bowden TJ (2016). Survey of potential reservoir species for the oyster parasite Multinucleate Sphere X (*Haplosporidium nelsoni*) in and around oyster farms in the Damariscotta River estuary, Maine. *Journal of Shellfish Research* 35: 851–856.

Messick GA (1994). *Hematodinium perezi* infections in adult and juvenile blue crabs *Callinectes sapidus* from coastal bays of Maryland and Virginia, USA. *Diseases of Aquatic Organisms* 19: 77−82.

Messick GA (1998). Diseases, parasites, and symbionts of blue crabs (*Callinectes sapidus*) dredged from Chesapeake Bay. *Journal of Crustacean Biology* 18: 533–548.

Messick GA, Small EB (1996). *Mesanophrys chesapeakensis* n. sp., a histophagous ciliate in the blue crab, *Callinectes sapidus*, and associated histopathology. *Invertebrate Biology* 115: 1–12.

Messick GA, Shields JD (2000). Epizootiology of the parasitic dinoflagellate *Hematodinium* sp. in the American blue crab *Callinectes sapidus. Diseases of Aquatic Organisms* 43: 139−152.

Metz DCG, Hechinger RF (2021). *Lynnia grapsolytica* n. gen, n. sp. (Ciliophora: Apostomatida: Colliniidae), a deadly blood parasite of crabs with a novel pseudocytopharynx. *The Journal of Eukaryotic Microbiology* 68: e12847 https://doi.org/10.1111/jeu.12847

Meyers TR, Koeneman TM, Bothelho C, Short S (1987). Bitter Crab Disease: a fatal dinoflagellate infection and marketing problem for Alaskan Tanner crabs *Chionoecetes bairdii*. *Diseases of Aquatic Organisms* 3: 195–216.

Meyers TR, Botelho C, Koeneman TM, Short S, Imamura K (1990). Distribution of bitter crab dinoflagellate syndrome in southeast Alaskan tanner crabs, *Chionoecetes bairdi*. *Diseases of Aquatic Organisms* 9: 37–43.

Meyers TR, Lightner DV, Redman R (1994). A dinoflagellate-like parasite in Alaskan spot shrimp *Pandalus platyceros* and pink shrimp *P. borealis*. *Diseases of Aquatic Organisms* 18: 71-76.

Meyers TR, Morado JF, Sparks AK, Bishop GH, Pearson T, Urban D, Jackson D (1996). Distribution of bitter crab syndrome in tanner crabs (*Chionoecetes bairdi, C. opilio*) from the Gulf of Alaska and the Bering Sea. *Diseases of Aquatic Organisms* 26: 221–227.

Midorikawa Y, Shimizu T, Sanda T, Hamasaki K, Dan S, Lal MTBM, Kato G, Sano M (2020). Characterization of *Aquimarina hainanensis* isolated from diseased mud crab *Scylla serrata* larvae in a hatchery. *Journal of Fish Diseases* 43: 541–549.

Miller TL, Small HJ, Peemoeller BJ, Gibbs DA, Shields JD (2013). Experimental infections of *Orchitophrya stellarum* (Scuticociliata) in American blue crabs (*Callinectes sapidus*) and fiddler crabs (*Uca minax*). *Journal of Invertebrate Pathology* 114: 346–355.

Molloy DP, Giambérini L, Stokes NA, Burreson EM, Ovcharenko MA (2012). *Haplosporidium raabei* n. sp. (Haplosporidia): a parasite of zebra mussels, *Dreissena polymorpha* (Pallas, 1771). *Parasitology* 139: 463−477.

Momoyama K, Hiraoka M, Nakano H, Koube H, Inouye K, Oseko N (1994). Mass mortalities of cultured kuruma shrimp *Penaeus japonicus*, in Japan in 1993: histopathological study. *Fish Pathology* 29: 141-148.

Momoyama K, Hiraoka M, Nakano H, Sameshima M (1998). Cryopreservation of penaeid rod-shaped DNA virus (PRDV) and its survival in sea water at different temperatures. *Fish Pathology* 33: 95–96.

Montgomery SS (1990). Movements of juvenile eastern king prawns *Penaeus plebejus*, and identification of stock along the east coast of Australia. *Fishery Research* 9: 189-208.

Montgomery-Fullerton MM, Cooper RA, Kauffman KM, Shields JD, Ratzlaff RE (2007). Detection of *Panulirus argus* Virus 1 in Caribbean spiny lobsters. *Diseases of Aquatic Organisms* 76: 1–6.

Moodie EG, Le Jambre LF, Katz ME (2003a). *Thelohania montirivulorum* sp. nov. (Microspora: Thelohaniidae), a parasite of the Australian freshwater crayfish, *Cherax destructor* (Decapoda: Parastacidae): fine ultrastructure, molecular characteristics and phylogenetic relationships. *Parasitology Research* 91: 215 -228.

Moodie EG, Le Jambre LF, Katz ME (2003b). Ultrastructural characteristics and small subunit ribosomal DNA sequence of *Vairimorpha cheracis* sp. nov., (Microspora: Burenellidae), a parasite of the Australian yabby, *Cherax destructor* (Decapoda: Parastacidae). *Journal of Invertebrate Pathology* 84: 198-213.

Moodie EG, Le Jambre LF, Katz ME (2003c). *Thelohania parastaci* sp. nov. (Microspora: Thelohaniidae), a parasite of the Australian freshwater crayfish, *Cherax destructor* (Decapoda: Parastacidae). *Parasitology Research* 91: 151-165.

Morado JF, Small EB (1994). Morphology and stomatogenesis of *Mesanophrys pugettensis* n. sp. (Scuticociliatida: Orchitophryidae), a facultative parasitic ciliate of the Dungeness crab, *Cancer magister* (Crustacea: Decapoda). *Transactions of the American Microscopical Society* 113: 343-364.

Morado JF, Small EB (1995). Ciliate parasites and related diseases of crustacea: a review. *Reviews in Fisheries Science* 3: 275–354.

Morado JF, Giesecke RH, Syrjala SE (1999). Molt-related mortalities of the Dungeness crab *Cancer magister* caused by a marine facultative ciliate *Mesanophrys pugettensis*. *Diseases of Aquatic Organisms* 38: 143-150.

Moss J, Butler MJ, Behringer DC, Shields JD (2012). Genetic diversity of the Caribbean spiny lobster virus, *Panulirus argus* virus 1 (PaV1), and the discovery of PaV1 in lobster postlarvae. *Aquatic Biology* 14: 223-232.

Moss J, Behringer D, Shields JD, Baeza A, Aguilar-Perera A, Bush PG, Dromer C, Herrera-Moreno H, Gittens L, Matthews TR, McCord MR, Schärer MT, Reynal L, TrueloveN, Butler MJ (2013). Distribution, prevalence, and genetic analysis of *Panulirus argus* virus 1 (PaV1) from the Caribbean Sea. *Diseases of Aquatic Organisms* 104: 129-140.

Mullen TE, Russell S, Tucker MT, Maratea JL, Koerting C, Hinckley L, De Guise S, Frasca S, French RA, Burrage TG, Perkins C (2004). Paramoebiasis associated with mass mortality of American lobster *Homarus americanus* in Long Island Sound, USA. *Journal of Aquatic Animal Health* 16: 29-38.

Munday BL, O'Donoghue PJ, Watts M, Rough K, Hawkesford T (1997). Fatal encephalitis due to the scuticociliate *Uronema nigricans* in sea-caged, southern bluefin tuna *Thunnus maccoyii*. *Diseases of Aquatic Organisms* 30: 17–25.

Murphy NE, Goggin CL (2000). Genetic discrimination of sacculinid parasites (Cirripedia, Rhizocephala): Implication for control of introduced green crabs (*Carcinus maenas*). *Journal of Crustacean Biology* 20: 153-157.

Musgrove RJ, Geddes MC, Thomas C (2005). Causes of tail fan necrosis in the southern rock lobster, *Jasus edwardsii*. *New Zealand Journal of Marine and Freshwater Research* 39: 293-304.

Musthaq SS, Sudhakaran R, Balasubramanian G, Sahul Hameed AS (2006). Experimental transmission and tissue tropism of white spot syndrome virus (WSSV) in two species of lobsters, *Panulirus homarus* and *Panulirus ornatus*. *Journal of Invertebrate Pathology* 93: 75–80.

Nakano H, Koube H, Umezawa S, Momoyama K, Hiraoka M, Inouye K, Oseko N (1994). Mass mortalities of cultured shrimp *Penaeus japonicus*, in Japan in 1993: epizootiological survey and infection trials. *Fish Pathology* 29: 135-139.

Nakano H, Hiraoka M, Sameshima M, Kimura T, Momoyama K (1998). Inactivation of penaeid rod-shaped DNA virus (PRDV), the causative agent of penaid acute viremia (PAV), by some chemical and physical treatments. *Fish Pathology* 33: 65-71.

Newman MW, Johnson CA (1975). A disease of blue crabs (*Callinectes sapidus*) caused by a parasitic dinoflagellate, *Hematodinium* sp. *Journal of Parasitology* 63: 554–557.

Newman MW, Johnson CA, Pauley GB (1976). A *Minchinia*-like haplosporidian parasitizing blue crabs, *Callinectes sapidus*. *Journal of Invertebrate Pathology* 27: 311–315.

Nilson EH, Fisher WS, Shleser RA (1976). A new mycosis of larval lobster (*Homarus americanus*). *Journal of Invertebrate Pathology* 27: 177–183.

Novotny MJ, Cawthorn RJ, Despres B (1996). *In-vitro* effects of chemotherapeutants on the lobster parasite *Anophryoides haemophila*. *Diseases of Aquatic Organisms* 24: 233-237.

Nunan LM, Poulos BT, Lightner DV (1998). The detection of White Spot Syndrome Virus (WSSV) and Yellow Head Virus (YHV) in imported commodity shrimp. *Aquaculture* 160: 19-30.

Nunan LM, Poulos BT, Redman M, Le Groumellec M, Lightner DV (2003a). Molecular detection methods developed for a systemic rickettsia-like bacterium (RLB) in *Penaeus monodon* (Decapoda: Crustacea). *Diseases of Aquatic Organisms* 53: 15-23.

Nunan LM, Noble B, Le Groumellec M, Lightner DV (2003b). Experimental infection of *Penaeus vannamei* by a rickettsia-like bacterium (RLB) originating from *Penaeus monodon*. *Diseases of Aquatic Organisms* 54: 43-48.

Nunan LM, Lightner DV, Pantoja CR, Stokes NA, Reece KS (2007). Characterization of a rediscovered haplosporidian parasite from cultured *Penaeus vannamei*. *Diseases of Aquatic Organisms* 74: 67–75.

Nunan LM, Poulos BT, Navarro S, Redman RM, Lightner DV (2010). Milky hemolymph syndrome (MHS) in spiny lobsters, penaeid shrimp and crabs. *Diseases of Aquatic Organisms* 91: 105-112.

Nur I, Yusnaini (2018). Parasites and histopathology of infected spiny lobster Panulirus spp. cultured in outer of Kendari Bay, Indonesia. *AACL Bioflux* 11: 108-117 http://www.bioflux.com.ro/docs/2018.108-117.pdf

Oakey HJ, Smith CS (2018). Complete genome sequence of a white spot syndrome virus associated with a disease incursion in Australia. *Aquaculture* 484: 152–159.

Oakey J, Smith C, Underwood D, Afsharnasab M, Alday-Sanz V, Dhar A, Sivakumar S, Sahul Hameed AS, Beattie K, Crook A (2019). Global distribution of white spot syndrome virus genotypes determined using a novel genotyping assay. *Archives of Virology* 164: 2061–2082.

O'Donoghue PJ, Adlard RD (2000). Catalogue of protozoan parasites recorded in Australia. *Memoirs of the Queensland Museum* 45: 1-163.

Oidtmann B, Stentiford GD (2011). White Spot Syndrome Virus (WSSV) concentrations in crustacean tissues – A review of data relevant to assess the risk associated with commodity trade. *Transboundary and Emerging Diseases* 58: 469–482.

OIE (2008). Milky haemolymph disease of spiny lobsters (*Panulirus* spp.). OIE Aquatic Animal Disease Cards, 2008. https://www.oie.int/app/uploads/2021/03/milky-20haemolymph-20disease-20of-20lobsters-20card-20-9-04-08-.pdf

OIE (2009) Aquatic animal health code, 12th edition. OIE–World Organization for Animal Health, Paris.

OIE (2021a). *Aquatic Animal Health Code*. Chapter 2.1 Import Risk Analysis. OIE Paris, France. https://www.oie.int/en/what-we-do/standards/codes-and-manuals/aquatic-code-online-access/?id=169&L=1&htmfile=chapitre_import_risk_analysis.htm

OIE (2021b). *Manual of Diagnostic Tests for Aquatic Animals* 2021. Chapter 2.2.8. Infection with White Spot Syndrome Virus. . https://www.oie.int/fileadmin/Home/eng/Health_standards/aahm/current/2.2.08_WSSV.pdf

Ooi MC, Goulden EF, Smith GG, Nowak BF, Bridle AR ((2017). Developmental and gut-related changes to the microbiomes of the cultured juvenile spiny lobster *Panulirus ornatus*. *FEMS Microbiology Ecology* 93: fix159.

Ooi M, Goulden EF, Trotter AJ, Smith GG, Bridle AR (2020). *Aquamarina* sp. associated with a cuticular disease of cultured larval Palinurid and Scyllarid lobsters. *Frontiers in Microbiology* 11: 573588.

Oseko N, Chuah TT, Maeno Y, Kua BC, Palanisamy V (2006). Examination for viral inactivation of WSSV (White Spot Syndrome Virus) isolated in Malaysia using Black Tiger Prawn (*Penaeus monodon*). *JARQ* 40: 93-97.

Otta SK, Patil PK, Jithendran KP, Rajendran KV, Alavandi SV, Vijayan KK (2016). Managing *Enterocytozoon hepatopenaei* (EHP), microsporidial infections in vannamei shrimp farming: An Advisory. CIBA e-publication No.29; January 2016. 8 pgs.

Overstreet RM, Whatley EC (1975). Prevention of microsporidiosis in the blue crab with notes on natural infections. In: Avault, J.W., Miller, R. (Eds.), Proceedings of the Sixth Annual Workshop World Mariculture Society. Louisiana State University, Louisiana, pgs. 335– 345.

Overstreet RM, Jovonovich J, Ma H (2009). Parasitic crustaceans as vectors of viruses, with an emphasis on three penaeid viruses. *Integrative and Comparative Biology* 49: 127–141.

Owens L (1997). Special topic review: the history of the emergence of viruses in Australian prawn aquaculture. *World Journal of Microbiology and Biotechnology* 13: 427-431.

Owens L, Glazebrook JS (1988). Microsporidosis in prawns from northern Australia. *Australian Journal of Marine and Freshwater Research* 39: 301-305.

Palenzuela O, Redondo MJ, Cali A, Takvorian PM, Alonso-Naveiro M, Alvarez-Pellitero P, Sitja-Bobadilla A (2014). A new intranuclear microsporidium, *Enterospora nucleophila* n. sp., causing an emaciative syndrome in a piscine host (*Sparus aurata*), prompts the redescription of the family Enterocytozoonidae. *International Journal of Parasitology* 44: 189-203.

Pascual-Jiménez C, Huchin-Mian JP, Simões N, Briones-Fourzán P, Lozano-Álvarez E, Sánchez Arteaga A, Antonio Pérez-Vega J, Simá-Álvarez R, Rosas Vazquez C, Rodríguez-Canul R (2012). Physiological and immunological characterization of Caribbean spiny lobsters *(Panulirus argus)* naturally infected with *Panulirus argus* Virus 1 (PaV1). *Diseases of Aquatic Organisms* 100: 113–124.

Payne MS, Hall MR, Bannister R, Sly L, Bourne DG (2006). Microbial diversity within the water column of a larval rearing system for the ornate rock lobster (*Panulirus ornatus*). *Aquaculture* 258: 80–90.

Payne MS, Høj L, Wietz M, Hall MR, Sly L, Bourne DG (2008). Microbial diversity of mid-stage Palinurid phyllosoma from Great Barrier Reef waters. *Journal of Applied Microbiology* 105: 340–350.

Paynter JL, Lightner DV, Lester RJG (1985). Prawn virus from juvenile *Penaeus esculentus*. In: Second Australian National Prawn Seminar, Cleveland, Australia ed Rothlisberg PC, Hill BJ, Staples DJ, pgs 61-64. http://www.frdc.com.au/Archived-Reports/FRDC%20Projects/1983-067%20Proceedings.pdf

Peng SE, Lo CF, Ho CH, Chang CF, Kou GH (1998). Detection of white spot baculovirus (WSBV) in giant freshwater prawn, *Macrobrachium rosenbergii*, using polymerase chain reaction. *Aquaculture* 164: 253-262.

Perera E, Simon C (2015). Digestive physiology of spiny lobsters: implications for formulated diet development. *Reviews in Aquaculture* 7: 243–261.

Perkins FO (1975). Fine-structure of *Minchinia* sp. (Haplosporida) sporulation in mud crab, *Panopeus herbstii*. Marine Fisheries Review 37(5-6): 46-60.

Pestal GP, Taylor DM, Hoenig JM, Shields JD, Pickavance R (2003). Monitoring the presence of the lethal parasite *Hematodinium* sp. in snow crabs from Newfoundland. *Diseases of Aquatic Organisms* 53: 67–75.

Petersen EH, Phuong TH (2011). Bioeconomic analysis of improved diets for lobster, *Panulirus ornatus*, culture in Vietnam. *Journal of the World Aquaculture Society* 42: 1-11.

Poisson R (1930). Observations sur *Anophrys sarcophagi* Cohn (=*A. maggii* Cattaneo) infusoire holotriche marin et sur son parasitisme possible chez certains Crustaceans. *Bulletin Biologique de la France et de la Belgique* 64: 288-331.

Porter L, Butler IV M., Reeves RH (2001). Normal bacterial flora of the spiny lobster *Panulirus argus* and its possible role in shell disease. *Marine and Freshwater Research* 52: 1401-1405.

Powell A, Scolding JW (2018). Direct application of ozone in aquaculture systems. *Reviews in Aquaculture* 10: 424-438.

Powell EN, Klinck JM, Ford SE, Hofmann EE, Jordan SJ (1999). Modeling the MSX parasite in eastern oyster (*Crassostrea virginica*) populations. III. Regional application and the problem of transmission. *Journal of Shellfish Research* 18: 517–537.

Pramod Kiran RB, Rajendran KV, Jung SJ, Oh MJ (2002). Experimental susceptibility of different lifestages of the giant freshwater prawn, *Macrobrachium rosenbergii* (de Man), to white spot syndrome virus (WSSV). *Journal of Fish Diseases* 25: 201-207.

Quinn RA, Metzler A, Smolowitz RM, Tlusty M, Chistoserdov AY (2012). Exposures of *Homarus americanus* shell to three bacteria isolated from naturally occurring epizootic shell disease lesions. *Journal of Shellfish Research* 31: 485-493.

Rahman MM, Corteel M, Wille M, Alday-Sanz V, Pensaert MB, Sorgeloos P, Nauwynck HJ (2007). The effect of raising water temperature to 33°C in *Penaeus vannamei* juveniles at different stages of infection with white spot syndrome virus (WSSV). *Aquaculture* 272: 240-245.

Raja K, Rahman MM, Rajkumar M, Gopalakrishnan A, Vijayakumara R (2015). Effect of ingestion and waterborne routes under different shrimp densities on white spot syndrome virus susceptibility in three commercially important penaeid shrimps. *Aquaculture Reports* 2: 120–125.

Rajendran KV, Vijayan KK, Santiago TC, Krol RM (1999). Experimental host range and histopathology of white spot syndrome virus (WSSV) infection in shrimp, prawns, crabs and lobsters from India. *Journal of Fish Diseases* 22: 183-91.

Rajendran KV, Shivam S, Ezhil Praveena P., Joseph Sahaya Rajan J, Sathish Kumar T, Avunje S, Jagadeesan V, Prasad Babu SV, Pande A, Navaneeth Krishnan A, Alavandi SV, Vijayan KK (2016). Emergence of *Enterocytozoon hepatopenaei* (EHP) in farmed *Penaeus (Litopenaeus) vannamei* in India, *Aquaculture* 454: 272-280.

Ranson HJ, LaPorte J, Spinard E, Chistoserdov AY, Gomez-Chiarri M., Nelson DR, Rowley DC (2018). Draft genome sequence of the putative marine pathogen *Aquimarina* sp. strain I32. 4. *Genome Announcements* 6: e00313–e00318.

Reardon KM, Wilson CJ, Gillevet PM, Sikaroodi M, Shields JD (2018). Increasing prevalence of epizootic shell disease in American lobster from the nearshore Gulf of Maine. *Bulletin of Marine Science* 94: 903–921.

Reddy AD, Jeyasekaran G, Shakila RJ (2011a). Effect of processing treatments on the white spot syndrome virus DNA in farmed shrimps (*Penaeus monodon*). *Letters in Applied Microbiology* 52: 393-398.

Reddy AD, Jeyasekaran G, Shakila RJ (2011b). White spot syndrome virus (WSSV) transmission risk through infected cooked shrimp products assessed by polymerase chain reaction and bio-inoculation studies. *Continental Journal of Fisheries and Aquatic Sciences* 5: 16-23.

Reddy AD, Jeyasekaran G, Shakila RJ (2013). Morphogenesis, Pathogenesis, Detection and Transmission Risks of White Spot Syndrome Virus in Shrimps. *Fisheries and Aquaculture Journal* 2013: FAJ-66.

Reece KS, Burreson EM, Bower S, Dungan CF (2000). Molecular analyses of a parasite in prawns (*Pandalus platyceros*) from British Columbia, Canada. *Journal of Shellfish Research* 19: 647.

Reece KS, Siddall ME, Stokes NA, Burreson EM (2004). Molecular phylogeny of the Haplosporidia based on two independent genes sequences. *Journal of Parasitology* 90: 1111–1122.

Renault T, Stokes NA, Chollet B, Cochennec N, Berthe F, Gerad A, Burreson EM (2000). Haplosporidosis in the Pacific oyster *Crassostrea gigas* from the French Atlantic coast. *Diseases of Aquatic Organisms* 42: 207-214.

Ritar AJ, Smith GG, Thomas CW (2006). Ozonation of seawater improves the survival of larval southern rock lobster, *Jasus edwardsii*, in culture from egg to juvenile. *Aquaculture* 261: 1014–1025.

Romero X, Turnbull JF, Jimenez R (2000). Ultrastructure and cytopathology of a Rickettsia-like Organism causing systemic infection in the redclaw crayfish, *Cherax quadricarinatus* (Crustacea: Decapoda), in Ecuador. *Journal of Invertebrate Pathology* 76: 95-104.

Ross EP, Behringer DC, Bojko J (2019a). White spot syndrome virus and the Caribbean spiny lobster, *Panulirus argus*: Susceptibility and behavioral immunity. *Journal of Invertebrate Pathology* 162: 1-9.

Ross EP, Behringer DC, Muñoz A, Díaz D, Bojko J (2019b). A histological atlas for the Palinuridae (Crustacea: Decapoda: Achelata): A guide to parasite discovery and spotting the abnormal in spiny lobsters. *Journal of Invertebrate Pathology* 163: 21–33.

Rowland SJ, Ingram BA (1991). Diseases of Australian indigenous freshwater fishes (with emphasis on the ectoparasitic and fungal diseases of Murray cod (*Macellochella peeli*, golden perch (*Macquaria ambigua*) and silver perch (*Bidyanus bidyanus*)). *New South Wales Fisheries Bulletin 4*: 33 pgs.

Ruello NV (1977). Migration and stock studies on the Australian school prawn *Metapenaeus macleayi*. *Marine Biology* 41: 185-190.

Ryazanova TV, Eliseikina MG, Kukhlevsky AD (2021). First detection of *Hematodinium* sp. in spiny king crab *Paralithodes brevipes*, and new geographic areas for the parasite in tanner crab *Chionoecetes bairdi*, and red king crab *Paralithodes camtschaticus*. *Journal of Invertebrate Pathology* 184: 107651.

Sahul-Hameed AS, Charles MX, Anilkumar M (2000). Tolerance of *Macrobrachium rosenbergii* to white spot syndrome virus. *Aquaculture* 183: 207-213.

Sahul-Hameed AS, Balasubramanian G, Musthaq SS, Yoganandhan K (2003). Experimental infection of twenty species of Indian marine crabs with white spot syndrome virus (WSSV). *Diseases of Aquatic Organisms* 57: 157-161.

Salachan PV, Jaroenlak P, Thitamadee S, Itsathitphaisarn O, Sritunyalucksana K (2017). Laboratory cohabitation challenge model for shrimp hepatopancreatic microsporidiosis (HPM) caused by *Enterocytozoon hepatopenaei* (EHP). *BMC Veterinary Research* 13: 9 doi: 10.1186/s12917-016-0923-1.

Santhoshkumar S, Sivakumar S, Vimal S, Abdul Majeed S, Taju G, Haribabu P, Uma A, Sahul Hameed AS (2016). Biochemical changes and tissue distribution of *Enterocytozoon hepatopenaei* (EHP) in

naturally and experimentally EHP-infected whiteleg shrimp, *Litopenaeus vannamei* (Boone, 1931), in India. *Journal of Fish Diseases* doi: 10.1111/jfd.12530.

Scott-Orr H, Jones JB, Bhatia N (2017). Uncooked prawn imports: effectiveness of biosecurity controls. Australian Government Inspector-General of Biosecurity Review report No. 2017–18/01. www.igb.gov.au/uncooked-prawn-imports-effectiveness-biosecurity-controls

Schapiro HC, Mathewson JH, Steenbergen JF, Kellogg S, Ingram C, Nierengarten G, Rabin H (1974). Gaffkemia in the California spiny lobster, *Panulirus interruptus*: Infection and immunization. *Aquaculture* 3: 403–408.

Senate (2017). Committee Hansard. Rural and Regional Affairs and Transport Legislation Committee. Estimates Tuesday 28th February 2017 . http://www.aph.gov.au/Parliamentary_Business/Hansard/Hansard_Display?bid=committees/estimate/d361919c-f8bb-4b70-a648-2e034c1d4d98/&sid=0000

Shaw RW, Kent ML, Adamson ML (2000). Viability of *Loma salmonae* (Microsporidia) under laboratory conditions. *Parasitology Research* 86: 978–981.

Shields JD (1992). Parasites and symbionts of the crab *Portunus pelagicus* from Moreton Bay, Eastern Australia. *Journal of Crustacean Biology* 12: 94-100.

Shields JD (1994). The parasitic dinoflagellates of marine crustaceans. *Annual Review of Fish Diseases* 4: 241–271.

Shields JD (2011). Diseases of spiny lobsters: A review. *Journal of Invertebrate Pathology* 106: 79–91.

Shields JD (2012). The impact of pathogens on exploited populations of decapod crustaceans. *Journal of Invertebrate Pathology* 110: 211-224.

Shields JD (2013). Complex etiologies of emerging diseases in lobsters (*Homarus americanus*) from Long Island Sound. *Canadian Journal of Fisheries and Aquatic Sciences* 70: 1576-1587.

Shields JD (2019). Climate change enhances disease processes in crustaceans: case studies in lobsters, crabs, and shrimps. *Journal of Crustacean Biology* 39: 673-683.

Shields JD, Kuris AM (1990). *Carcinonemertes wickhami* n. sp. (Nemertea), an egg predator on the California lobster, *Panulirus interruptus*. *Fisheries Bulletin* 88: 279–287.

Shields JD, Wood FEI (1993). Impact of parasites on the reproduction and fecundity of the blue sand crab *Portunus pelagicus* from Moreton Bay, Australia. *Marine Ecology Progress Series* 92: 159-170.

Shields JD, Behringer DC (2004). A pathogenic herpes-like virus in the Caribbean spiny lobster, *Panulirus argus*, from the Florida Key. *Diseases of Aquatic Organisms* 59: 109–118.

Shields JD, Taylor DM, Sutton SG, O'Keefe PO, Collins PW, Ings DW, Pardy AL (2005). Epizootiology of bitter crab disease (*Hematodinium* sp.) in snow crabs, *Chionoecetes opilio*, from Newfoundland, Canada. *Diseases of Aquatic Organisms* 64: 253–264.

Shields JD, Stephens FJ, Jones JB (2006). Chapter 5, Pathogens, Parasites and Other Symbionts. In: Phillips BF (Ed.), Lobsters, Biology, Management, Aquaculture and Fisheries. Blackwell Scientific, UK, pgs. 146–204.

Siemens (2011). Required UV Dose. Siemens Water Technologies UV datasheet. www.siemens.com/uv-disinfection WT.090.300.013.IE.DS.0108.

Small HJ (2012). Advances in our understanding of the global diversity and distribution of *Hematodinium* spp. – Significant pathogens of commercially exploited crustaceans. *Journal of Invertebrate Pathology* 110: 234–246.

Small EB, Lynn DH (1985). Phylum Ciliophora Doflein, 1901. In: Lee JJ, Hutner SH, Bovee EC (eds) *An Illustrated Guide to the Protozoa*. pgs 393-575. Society of Protozoologists, Lawrence, KS.

Small HJ, Neil DM, Taylor AC, Bateman K, Coombs GH (2005a). A parasitic scuticociliate infection in the Norway lobster (*Nephrops norvegicus*). *Journal of Invertebrate Pathology* 90: 108–117.

Small HJ, Neil DM, Taylor AC, Coombs GH (2005b). Identification and partial characterisation of metalloproteases secreted by a *Mesanophrys*-like ciliate parasite of the Norway lobster *Nephrops norvegicus*. *Diseases of Aquatic Organisms* 67: 225–231.

Small HJ, Shields JD, Hudson KL, Reece KS (2007). Molecular detection of *Hematodinium* sp. infecting the blue crab, *Callinectes sapidus*. *Journal of Shellfish Research* 26: 131–139.

Small HJ, Shields JD, Reece KS, Bateman K, Stentiford GD (2012). Morphological and molecular characterization of *Hematodinium perezi* (Dinophyceae: Syndiniales), a dinoflagellate parasite of the harbour crab, *Liocarcinus depurator*. *Journal of Eukaryotic Microbiology* 59: 54–66.

Small HJ, Miller TL, Huntley AH, Delaney KL, Schott E, Shields JD (2013). Discovery of an opportunistic starfish pathogen, *Orchitophrya stellarum*, in captive blue crabs, *Callinectes sapidus*. *Journal of Invertebrate Pathology* 114: 178–185.

Small HJ, Huchin-Mian JP, Reece KS, Pagenkopp Lohan KM, Butler MJ, Shields JD (2019a). Parasitic dinoflagellate *Hematodinium perezi* prevalence in larval and juvenile blue crabs *Callinectes sapidus* from coastal bays of Virginia. *Diseases of Aquatic Organisms* 134: 215-222.

Small HJ, Stentiford GD, Behringer DC, Freeman MA, Atherley NAM, Reece KS, Bateman KS, Shields JD (2019b). Characterization of microsporidian *Ameson herrnkindi* sp. nov. infecting Caribbean spiny lobsters *Panulirus argus*. *Diseases of Aquatic Organisms* 136: 209–218.

Smith PJ, McVeagh SM, Hulston D, Anderson SA, Gublin Y (2009). DNA identification of ciliates associated with disease outbreaks in a New Zealand marine fish hatchery. *Diseases of Aquatic Organisms* 86: 163-167.

Snowden KF (2004). Chapter 8. Zoonotic microsporidia from animals and arthropods with a discussion of human infections. In Lindsay DS, Weiss LM (eds). Opportunistic Infections: *Toxoplasma*, *Sarcocystis*, and Microsporidia. Kulwer Academic Publishers, Boston. pp 123-134.

Soto MA, Lotz JM (2001). Epidemiological parameters of white spot syndrome virus infections in *Litopenaeus vannamei* and *L. setiferus*. *Journal of Invertebrate Pathology* 78: 9-15.

Soto MA, Shervette VR, Lotz JM (2001). Transmission of white spot syndrome virus (WSSV) to *Litopenaeus vannamei* from infected cephalothorax, abdomen, or whole shrimp cadaver. *Diseases of Aquatic Organisms* 45: 81–87.

Spann KM, Lester RJG (1996). Baculovirus of *Metapenaeus bennettae* from the Moreton Bay region of Australia. *Diseases of Aquatic Organisms* 27: 53-58.

Sparks AK, Hibbits J, Fegley J (1982). Observations on the histopathology of a systemic ciliate (*Paranophrys* sp.) disease in the Dungeness crab, *Cancer magister*. *Journal of Invertebrate Pathology* 39: 219-228.

Sprague V (1963). *Minchinia louisiana* n. sp. (Haplosporidia, Haplosporidiidae), a parasite of *Panopeus herbstii*. *The Journal of Protozoology* 10: 267–274.

Sritunyalucksana K, Sanguanrut P, Salachan PV, Thitamadee S, Flegel TW (2014). Urgent appeal to control spread of the shrimp microsporidian parasite *Enterocytozoon hepatopenaei* (EHP). https://enaca.org/publications/health/disease-cards/urgent-appeal-to-control-ehp-3.pdf

Stentiford GD (2012). Diseases in aquatic crustaceans: Problems and solutions for global food security. *Journal of Invertebrate Pathology* 110: 139.

Stentiford GD, Shields JD (2005). A review of the parasitic dinoflagellates *Hematodinium* species and *Hematodinium*-like infections in marine crustaceans. *Diseases of Aquatic Organisms* 66: 47-70.

Stentiford GD, Neil DM (2011). Diseases of *Nephrops* and *Metanephrops*: A review. *Journal of Invertebrate Pathology* 106: 92–109.

Stentiford GD, Green M, Bateman K, Small HJ, Neil DM, Feist SW (2002). Infection by a *Hematodinium*-like parasitic dinoflagellate causes Pink Crab Disease (PCD) in the edible crab *Cancer pagurus*. *Journal of Invertebrate Pathology* 79: 179–191.

Stentiford GD, Feist SW, Bateman KS, Hine PM (2004). Haemolymph parasite of the shore crab *Carcinus maenas*: pathology, ultrastructure and observations on crustacean haplosporidians. *Diseases of Aquatic Organisms* 59: 57-68.

Stentiford GD, Bateman KS, Feist SW (2007). *Enterospora canceri* n.gen., n.sp., an intranuclear microsporidian infecting European edible crab (*Cancer pagurus*). *Diseases of Aquatic Organisms* 75: 61–72.

Stentiford GD, Bonami JR, Alday-Sanz V (2009). A critical review of susceptibility of crustaceans to taura Syndrome, Yellowhead disease and White Spot Disease and implications of inclusion of these diseases in European legislation. *Aquaculture* 291: 1-17.

Stentiford GD, Bateman KS, Small HJ, Moss J, Shields JD, Reece KS, Tuck I (2010). *Myospora metanephrops* (n. g., n. sp.) from marine lobsters and a proposal for erection of a new order and family

(Crustaceacida; Myosporidae) in the Class *Marinosporidia* (Phylum Microsporidia). *International Journal for Parasitology* 40: 1433–1446.

Stentiford GD, Bateman KS, Dubuffet A, Chambers E, Stone DM (2011). *Hepatospora eriocheir* (Wang and Chen, 2007) gen. et comb. nov. infecting invasive Chinese mitten crabs (*Eriocheir sinensis*) in Europe. *Journal of Invertebrate Pathology* 108: 156–166.

Stentiford GD, Neil DM, Peeler EJ, Shields JD, Small HJ, Flegel TW, Vlak JM, Jones JB, Morado F, Moss S, Lotz J, Bartholomay L, Behringer DC, Hauton C, Lightner DV (2012). Disease will limit future food supply from the global crustacean fishery and aquaculture sectors. *Journal of Invertebrate Pathology* 110: 141–157.

Stentiford GD, Bateman KS, Stokes NA, Carnegie RB (2013). *Haplosporidium littoralis* sp. nov.: a crustacean pathogen within the Haplosporida (Cercozoa, Ascetosporea). *Diseases of Aquatic Organisms* 105: 243-252.

Stentiford GD, Neil DM, Albalat A, Milligan RJ, Bailey N (2015). The effect of parasitic infection by *Hematodinium* sp. on escape swimming and subsequent recovery in the Norway lobster *Nephrops norvegicus* (L.). *Journal of Crustacean Biology* 35: 1-10.

Stentiford GD, Becnel JJ, Weiss LM, Keeling PJ, Didier ES, Williams BA, Bjornson S, Kent ML, Freeman MA, Brown MJ, Troemel ER, Roesel K, Sokolova Y, Snowden KF, Solter L (2016). Microsporidia – Emergent pathogens in the global food chain. *Trends in Parasitology* 32: 336–348.

Stentiford GD, Ross S, Minardi D, Feist SW, Bateman KS, Gainey PA, Troman C, Bass D (2018). Evidence for trophic transfer of *Inodosporus octospora* and *Ovipleistophora arlo* n. sp. (Microsporidia) between crustacean and fish hosts. *Parasitology* 145: 1105–1117.

Stephens F, Fotedar S, Evans L (2003). Rock lobster health and diseases: A guide for the lobster industry. Curtin University of Technology, Aquatic Science Research Unit, ISBN: 1740672917. 28 pgs.

Stewart JE (1975). Gaffkemia, the fatal infection of lobsters (genus *Homarus*) caused by *Aerococcus viridans* (var.) *homari*: a review. *Marine Fisheries Review* 37(5-6): 20-24.

Sub-Committee on Aquatic Animal Health (SCAAH) (2017). Aquaculture Farm Biosecurity Plan: generic guidelines and template. Department of Agriculture and Water Resources, Canberra, January 2017. 40 pgs

Subramaniam K, Behringer DC, Bojko J, Yutin N, Clark AS, Bateman KS, van Aerle R, Bass D, Kerr RC, Koonin EV, Stentiford GD, Waltzek TB (2020). A new family of DNA viruses causing disease in crustaceans from diverse aquatic biomes. *mBio* 11:e02938-19. https://doi.org/10.1128/mBio.02938-19

Sudewi, Widiastuti Z, Salmet B, Mahardika K (2018a). Investigation of diseases in grow out of spiny lobster *Panulirus homarus* cultured in floating net cages (Lombok, Pegametan and Pangandaran). *Jurnal Ilmu dan Teknologi Kelautan Tropis* 10: 111-122.

Sudewi, Widiastuti Z, Salmet B, Mahardika K (2018b). Experimental infections of milky hemolymph disease in spiny lobster *Panulirus homarus*. *Indonesian Aquaculture Journal* 13: 31-40.

Sudewi, Widiastuti Z, Wardana IK, Mahardika K (2020). Molecular detection and phylogenetic of Milky Hemolymph Disease: Case from different locations in Indonesia. *Journal of Aquaculture and Fish Health* 9: 48-56.

Summerfelt ST (2003). Ozonation and UV irradiation - an introduction and examples of current applications. *Aquacultural Engineering* 28: 21-36.

Sunila I, Karolus J, Lang EP, Mroczka ME, Volk J (2000). Transmission of the haplosporidian parasite *Haplosporidium nelsoni* to the eastern oyster *Crassostrea virginica* in an upweller system. *Diseases of Aquatic Organisms* 42: 153-155

Supamattaya K, Hoffmann RW, Boonyaratpalin S, Kanchanaphum P (1998). Experimental transmission of white spot syndrome virus (WSSV) from black tiger shrimp *Penaeus monodon* to the sand crab *Portunus pelagicus*, mud crab *Scylla serrata* and krill *Acetes sp*. *Diseases of Aquatic Organisms* 32: 79-85.

Takahashi Y, Itami T, Kondom M, Maeda M, Fuji R, Tomonaga S, Supamattaya K, Boonyaratpalin S (1994). Electron microscopic evidence of baciliform virus infection in Kuruma shrimp (*Penaeus japonicus*). *Fish Pathology* 29: 121–125.

Tan CK, Owens L (2000). Infectivity, transmission and 16s rRNA sequencing of a rickettsia, *Coxiella cheraxi* sp. nov., from the freshwater crayfish *Cherax quadricarinatus*. *Diseases of Aquatic Orgamisms* 41: 115-122.

Tang KF, Pantoja CR, Redman RM, Han JE, Tran LH, Lightner DV (2015). Development of *in situ* hybridization and PCR assays for the detection of *Enterocytozoon hepatopenaei* (EHP), a microsporidian parasite infecting penaeid shrimp. *Journal of Invertebrate Pathology* 130: 37–41.

Tang KF, Han JE, Aranguren LF, White-Noble B, Schmidt MM, Piamsomboon P, Risdiana E, Hanggono B (2016). Dense populations of the microsporidian *Enterocytozoon hepatopenaei* (EHP) in feces of *Penaeus vannamei* exhibiting white feces syndrome and pathways of their transmission to healthy shrimp. *Journal of Invertebrate Pathology* 140: 1–7.

Thitamadee, S, Prachumwat A, Srisala J, Jaroenlak P, Salachan PV, Sritunyalucksana K, Flegel TW, Itsathitphaisarn O (2016). Review of current disease threats for cultivated penaeid shrimp in Asia. *Aquaculture* 452: 69–87.

Tourtip S, Tourtip S, Wongtripop S, Sritunyalucksana K, Stentiford GD, Bateman KS, Sriurairatana S, Chayaburakul K, Chavadej K, Withyachumnarnkul B (2009). *Enterocytozoon hepatopenaei* sp. nov. (Microspora: Enterocytozoonidae), a parasite of the black tiger shrimp *Penaeus monodon* (Decapoda: Penaeidae): fine structure and phylogenetic relationships. *Journal of Invertebrate Pathology* 102: 21–29.

Urrutia A., Bass D, Ward G, Ross S, Bojko J, Marigomez I., Feist SW (2019). Ultrastructure, phylogeny and histopathology of two novel haplosporidians parasitising amphipods, and importance of crustaceans as hosts. *Diseases of Aquatic Organisms* 136: 87-103.

Utari HB, Senapin S, Jaengsanong C., Flegel TW, Kruatrachue M (2012). A haplosporidian parasite associated with high mortality and slow growth in *Penaeus* (*Litopenaeus*) *vannamei* cultured in Indonesia. *Aquaculture* 366–367: 85-89.

Vidal-Martinez VM, Jimenez-Cueto AM, Sima-Alvarez R (2002). Parasites and symbionts of native and cultured shrimps from Yucatan, Mexico. *Journal of Aquatic Animal Health* 14: 57-64.

Walker G (2001). Introduction to the Rhizocephala (Crustacea: Cirripedia). *Journal of Morphology* 249: 1–8.

Walker AN, Lee RF, Frischer ME (2009). Transmission of the parasitic dinoflagellate *Hematodinium* sp. infection in blue crabs *Callinectes sapidus* by cannibalism. *Diseases of Aquatic Organisms* 85: 193−197.

Wang W, Chen J (2007). Ultrastructural study on a novel microsporidian, *Endoreticulatus eriocheir* sp. nov. (Microsporidia, Encephalitozoonidae), parasite of Chinese mitten crab, *Eriochier sinensis* (Crustacea, Decapoda). *Journal of Invertebrate Pathology* 94: 77–83.

Wang YC, Lo CF, Chang PS, Kou GH (1998). Experimental infection of white spot baculovirus in some cultured and wild decapods in Taiwan. *Aquaculture* 164: 221-31.

Wang YG, Lee KL, Najiah M, Shariff M, Hassan MD (2000). A new bacterial white spot syndrome (BWSS) in cultured tiger shrimp *Penaeus monodon* and its comparison with white spot syndrome (WSS) caused by virus. *Diseases of Aquatic Organisms* 41: 9-18.

Wang Z, Lu X, Liang Y, Wang C (2010). *Haplosporidium nelsoni* and *H. costale* in the Pacific oyster *Crassostrea gigas* from Chile's coasts. *Diseases of Aquatic Organisms* 89: 223–228.

Wang JF, Li M, Xiao J, Xu WJ, Li CW (2017). *Hematodinium* spp. infections in wild and cultured populations of marine crustaceans along the coast of China. *Diseases of Aquatic Organisms* 124: 181-191.

Ward GM, Feist SW, Noguera P, Marcos-López M, Ross S, Green M, Urrutia A, Bignell JP, Bass D (2019). Detection and characterisation of haplosporidian parasites of the blue mussel *Mytilus edulis*, including description of the novel parasite *Minchinia mytili* n. sp. *Diseases of Aquatic Organisms* 133: 57-68.

Webster NS, Bourne DG, Hall M (2006). *Vibrionaceae* infection in phyllosomas of the tropical rock lobster *Panulirus ornatus* as detected by fluorescence in situ hybridisation. *Aquaculture* 255: 173–178.

Weng HT (1987). The parasitic barnacle, *Sacculina granifera* Boschma, affecting the commercial sand crab, *Portunus pelagicus* (L.), in populations from two different environments in Queensland. *Journal of Fish Diseases* 10: 221-227.

Wilcox C, Hardesty B, Sharples R, Griffin D., Lawson T, Gunn R (2013). Ghostnet impacts on globally threatened turtles, a spatial risk analysis for northern Australia. *Conservation Letters* 6: 247–254.

Wilhelm G, Boulo V (1988). Infection de l'etrille *Liocarcinus puber* (L.) par un dinoflagellate parasite: *Hematodinium* sp. *Cons. Int. Explor. Mer. Ser.* CM K: 32: 1–10.

Wilhelm G, Mialhe E (1996). Dinoflagellate infection associated with the decline of *Necora puber* crab populations in France. *Diseases of Aquatic Organisms* 26: 213–219.

Williams JD, Boyko CB, Rice ME, Young CM (2019). A report on two large collections of the squat lobster *Munidopsis platirostris* (Decapoda, Anomura, Munidopsidae) from the Caribbean, with notes on their parasites, associates, and reproduction. *Journal of Natural History* 53: 159-169.

Winters AD, Faisal M (2014). Molecular and ultrastructural characterization of *Haplosporidium diporeiae* n. sp., a parasite of *Diporeia* sp. (Amphipoda, Gammaridea) in the Laurentian Great Lakes (USA). *Parasites and Vectors* 7: 343.

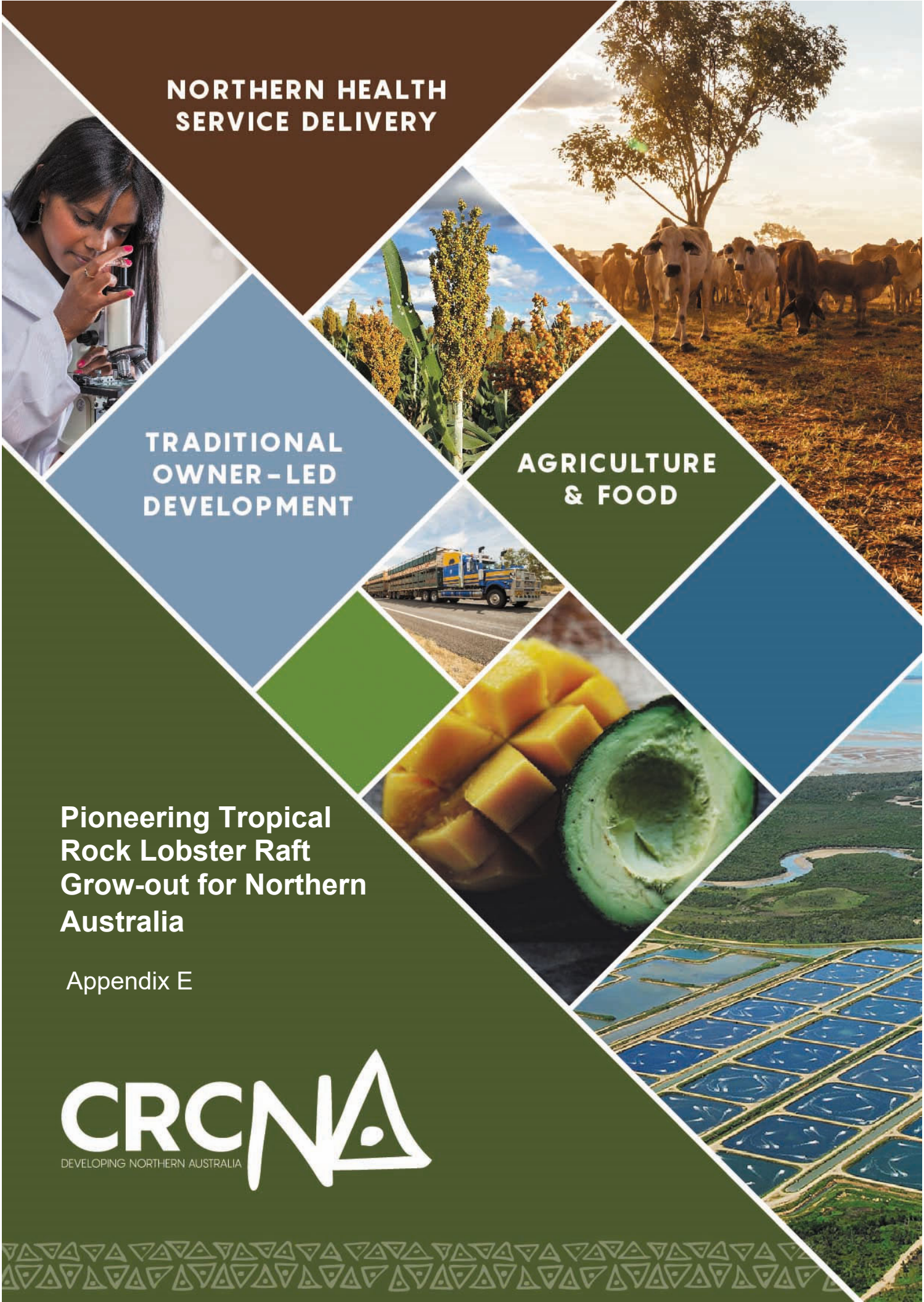
Wu JL, Namikoshi A, Nishizawa T, Mushiake K, Teruya K, Muroga K (2001). Effects of shrimp density on transmission of penaeid acute viremia in *Penaeus japonicus* by cannibalism and the waterborne route. *Diseases of Aquatic Organisms* 47: 129–135

Xu W, Xie J, Shi H, Li C (2010). *Hematodinium* infections in cultured ridgetail white prawns, *Exopalaemon carinicauda*, in eastern China. *Aquaculture* 300: 25-31.

Zamora-Briseno JA, Cerqueda-García D, Hernández-Velázqueza IM, Rivera-Bustamante R, Huchín-Mian JP, Briones-Fourzán P, Lozano-Álvarez E, Rodríguez-Canula R (2020). Alterations in the gut-associated microbiota of juvenile Caribbean spiny lobsters *Panulirus argus* (Latreille, 1804) infected with PaV1. *Journal of Invertebrate Pathology* 176: 107457.

Zha H, Jones B, Lewis G, Dong Y, Jeffs A (2018). Pathology of tail fan necrosis in the spiny lobster, *Jasus edwardsii*. *Journal of Invertebrate Pathology* 154: 5–11.

Zheng Y, Wang Y, Liu Y, Li W, Yu M, Zhang XH. (2016). *Aquimarina hainanensis* sp. nov., isolated from diseased Pacific white shrimp *Litopenaeus vannamei* larvae. *International Journal of Systematic and Evolutionary Microbiology* 66: 70–75.



Pioneering Tropical Rock Lobster Raft Grow-out for Northern Australia

Appendix E

CRCNA
DEVELOPING NORTHERN AUSTRALIA

Department of
Primary Industries and
Regional Development

GOVERNMENT OF
WESTERN AUSTRALIA

Health protocol for the import of tropical rock lobster (*Panulirus ornatus*) juveniles produced by a Queensland hatchery into Western Australia

Version 1.0 – 02 November 2022

Version history	Date	Document Reference
0.1 – Consultation draft	12 April 2022	A9329156
1.0	02 November 2022	A9740558

Table of Contents

Purpose

This health protocol is designed to minimise the risk of introducing priority diseases of concern[1] into Western Australia (WA) via Tropical rock lobster (*Panulirus ornatus* or TRL) juveniles produced by the source hatchery in Queensland.

Scope

This health protocol only applies to managing the disease risks associated with import of TRL juveniles from the source hatchery. It does not apply to other potential biosecurity or translocation risks, which are considered through other mechanisms.

This health protocol applies to TRL juveniles that are produced by the following source hatchery:

Ornatas Pty Ltd, Toomulla Beach, Qld, 4816

TRL juveniles produced under this protocol may only be shipped to Maxima Rock Lobster Pty Ltd, under the terms of their exemption to undertake research and development trials at Cone Bay, WA.

Where this protocol is referenced as a condition of a permit to import a potential carrier, under the *Biosecurity and Agriculture Management Act 2007*, the conditions of this protocol must be complied with. Failure to comply may result in a fine or other regulatory action.

Definitions

Approved laboratory A veterinary diagnostic laboratory that holds National Association of Testing Authorities (NATA) accreditation in Animal Health and is approved by the jurisdictional Chief Veterinary Officer (as required) to conduct testing for reportable/notifiable diseases (e.g. Biosecurity Sciences Laboratory Queensland).

Closed hatchery system An enclosed land-based facility for cultivation of lobster larvae and juveniles with biosecurity controls in place to ensure lobsters are at no time placed in, or exposed to, unfiltered and untreated Qld waters.

Competent authority The Queensland Department of Agriculture and Fisheries, including the Biosecurity Sciences Laboratory (BSL).

Independent certifier An appropriately qualified independent third-party certifier approved by the WA Chief Veterinary Officer.

Larval and juvenile production cycle The time from spawning of lobster broodstock within a closed hatchery system, to cultivation of larvae and juveniles prior to importation into WA.

[1] Priority diseases of concern identified in the Pathogen Risk Analysis for Aquaculture Biosecurity and Translocation of Tropical Rock Lobsters (*Panulirus ornatus*) in Northern Australia, DigsFish Services Pty Ltd, Final Version 29 November 2021.

Destination exemption holder The person who holds the WA exemption to undertake research and development trials that describes the site(s) on which the TRL juveniles will be placed.

Source Hatchery
Ornatas Pty Ltd, Toomulla Beach, Qld, 4816

Protocol

1. Source hatchery general requirements for production of TRL juveniles for WA

1.1. The hatchery must maintain a comprehensive hatchery biosecurity plan that is consistent with the national Aquaculture Farm Biosecurity Plan: generic guidelines and template[2]. The hatchery biosecurity plan must be available for audit and contain all relevant operational procedures including, but not limited to:

- broodstock management
- treatment of influent water
- disinfection methods, including procedure for between batch cleaning and disinfection
- process for notification and investigation of unusual or unexplained mortalities or signs of disease according to the requirements of the competent authority, and
- sampling for diagnostic testing.

1.2. *Panulirus ornatus* and *Artemia* spp. are the only species that are held in captivity at the source hatchery.

1.3. All seawater used in the hatchery system must be subject to nominal filtration 10 µm or less followed by foam fractionation and ozonation to an oxidation reduction potential (ORP) of between 700 and 800 mv (c. 1 - 1.5 mg/L) for 10 minutes equivalent to an approximate ozone dose of contact time (Ct) 10-15mg/L/min total residual oxidants.

1.4. Standard operating procedures and maintenance records for filtration and ozonation of seawater must be kept and made available for auditing by the competent authority or independent certifier.

1.5. Prior to the first TRL juvenile batch being imported to WA, and thereafter within the previous 12 months before import, the competent authority or independent certifier must audit the hatchery and certify in writing that the source hatchery adheres to, or is able to adhere to, the conditions outlined in sections 1 to 3 of this protocol.

[2] Sub-Committee on Aquatic Animal Health (SCAAH) 2016. Aquaculture Farm Biosecurity Plan: generic guidelines and template. Department of Agriculture and Water Resources, Canberra, https://www.awe.gov.au/agriculture-land/animal/aquatic/guidelines-and-resources.

1.6. Upon request the hatchery must provide hatchery access to the competent authority or independent certifier to conduct an audit.

2. Source hatchery TRL broodstock requirements

2.1. Incoming wild-caught broodstock must be held in a separate quarantine room for 6 weeks before being moved to the broodstock conditioning room.

2.2. Before exiting quarantine, wild-caught broodstock must be PCR tested negative for WSSV using non-destructive testing of haemolymph and/or pleopods.

2.3. The broodstock in the quarantine room must remain completely isolated from all other invertebrates (including TRL) at the hatchery, in a separate room with separate equipment and a separate supply of seawater treated as outlined in section 1.3 of this protocol.

2.4. Broodstock in the conditioning room must remain completed isolated from all other invertebrates (including TRL) at the hatchery, in a separate room with separate equipment and a separate supply of seawater treated as outlined in section 1.3 of this protocol.

2.5. Broodstock must be maintained in the quarantine and then conditioning systems for at least 6 months (combined time) prior to use for the production of larvae for the hatchery.

2.6. Broodstock must only be fed crustacean-free manufactured/processed feed, or supplemental natural feeds that are heat treated (minimum 70°C for 5 minutes, or 100°C >1 minute) or gamma irradiated to 50 kilogray (kGy) gamma irradiation.

2.7. Unusual or unexplained mortality rates or signs of disease in broodstock must be reported in accordance with requirements of the competent authority, and must be subject to laboratory investigation by the competent authority.

2.8. Broodstock at the end of their working life must be submitted to the competent authority or approved laboratory for relevant testing including histopathology. Pathology reports must be retained and provided to DPIRD on request, to assist in the review of biosecurity risk assessments for TRL translocation.

3. Source hatchery requirements for production of TRL juveniles for WA

3.1. Brooding lobsters must be separated from their hatching clutch as soon as possible.

3.2. The hatched larvae must be transferred from the broodstock conditioning rooms to the hatchery/nursery building as soon as possible.

3.3. All batches of larvae, puerulus or juveniles for WA must remain completely isolated from any other TRL at the source hatchery that do not meet the requirements of this protocol. Complete isolation requires batches to remain

in a separate room(s) with separate equipment and a separate supply of seawater treated as outlined in section 1.3 of this protocol. Where batches all meet the same requirements of the WA health protocol, those batches may be held in independent systems with separate equipment, but in adjacent tanks in the same room.

3.4. All juveniles destined for WA must be hatched and reared at the source hatchery in accordance with this protocol, from broodstock held at the source hatchery in accordance with this protocol.

3.5. Larvae and juveniles must only be fed certified disease (white spot syndrome virus)-free *Artemia* or a manufactured/processed feed.

3.6. A daily record of mortality must be maintained from day 1 of larval culture up to the day of dispatch of juveniles for translocation to WA.

3.7. Unusual or unexplained mortality rates or signs of disease in lobsters during the larval and juvenile production cycle must be reported in accordance with the requirements of the competent authority, and subject to laboratory investigation by the competent authority. Unusual or unexplained mortality includes mortality exceeding 10% of the total batch population in a 24h period, but is not limited to that trigger.

4. Pre-dispatch diagnostic testing of juveniles

4.1. At the completion of the larval and juvenile production cycle, a sample of at least 150 juvenile TRL (or as approved by DPIRD[3]) must be collected to allow for testing as outlined below. Each sample must be comprised of an equal number of juveniles randomly collected from each tank used to produce the batch for translocation to WA.

4.2. 150 juveniles must be tested by the competent authority or approved laboratory using the real time CSIRO WSSV TaqMan assay for the presence of white spot syndrome virus (WSSV).

4.3. 150 juveniles must be examined by the competent authority by histopathology for signs of disease including reportable/notifiable aquatic animal diseases

4.4. Prior to submission, the hatchery should contact the testing laboratory to discuss appropriate sampling to minimise the number of juveniles to be collected. For example, where possible, the same 150 juveniles may be used for PCR and histopathology testing, if individuals are cut in half along the sagittal plane with one half fixed in 80% ethanol for PCR testing, and the other half fixed in Davidson's solution for histology.

[3] A sample of 150 juveniles is approximated from the number of animals that must be sampled from a population 5000 or more, to demonstrate freedom from disease at the 95% level of confidence if disease is present at the minimum expected prevalence of 2% (and assuming perfect test sensitivity). *If there are less than 5000 juveniles in the batch of juvenile TRL, DPIRD may approve a reduction in the sample number in accordance with the sample size calculations for populations less than 5000.*

4.5. A laboratory pathology report/certificate must be provided by the competent authority indicating the absence of disease in the juveniles that have been PCR-tested and examined histologically, including the absence of reportable/notifiable aquatic animal diseases applicable to crustaceans listed under WA's *Biosecurity and Agriculture Management Act 2007*.

4.6. The laboratory pathology report/certificate must be dated no more than 30 days before shipment.

5. Pre-dispatch documentation to be provided to DPIRD

5.1. The source hatchery must provide the pre-dispatch documentation outlined below to DPIRD at least two working days before the shipment date, by emailing it to rob.gurney@dpird.wa.gov.au, and livestockbiosecurity@dpird.wa.gov.au.

5.2. A declaration signed by the source hatchery attesting that:

- The juveniles to be translocated adhere with this protocol (points 1 to 4 above)
- There have been no unusual or unexplained rates of mortality in the batch of juveniles to be translocated. The daily mortality log must be attached as evidence.
- The hatchery has not experienced any significant disease outbreaks including reportable/notifiable diseases in the previous 12 months.

5.3. The laboratory report/certificate of disease status for the batch to be translocated.

5.4. The current hatchery biosecurity audit certificate.

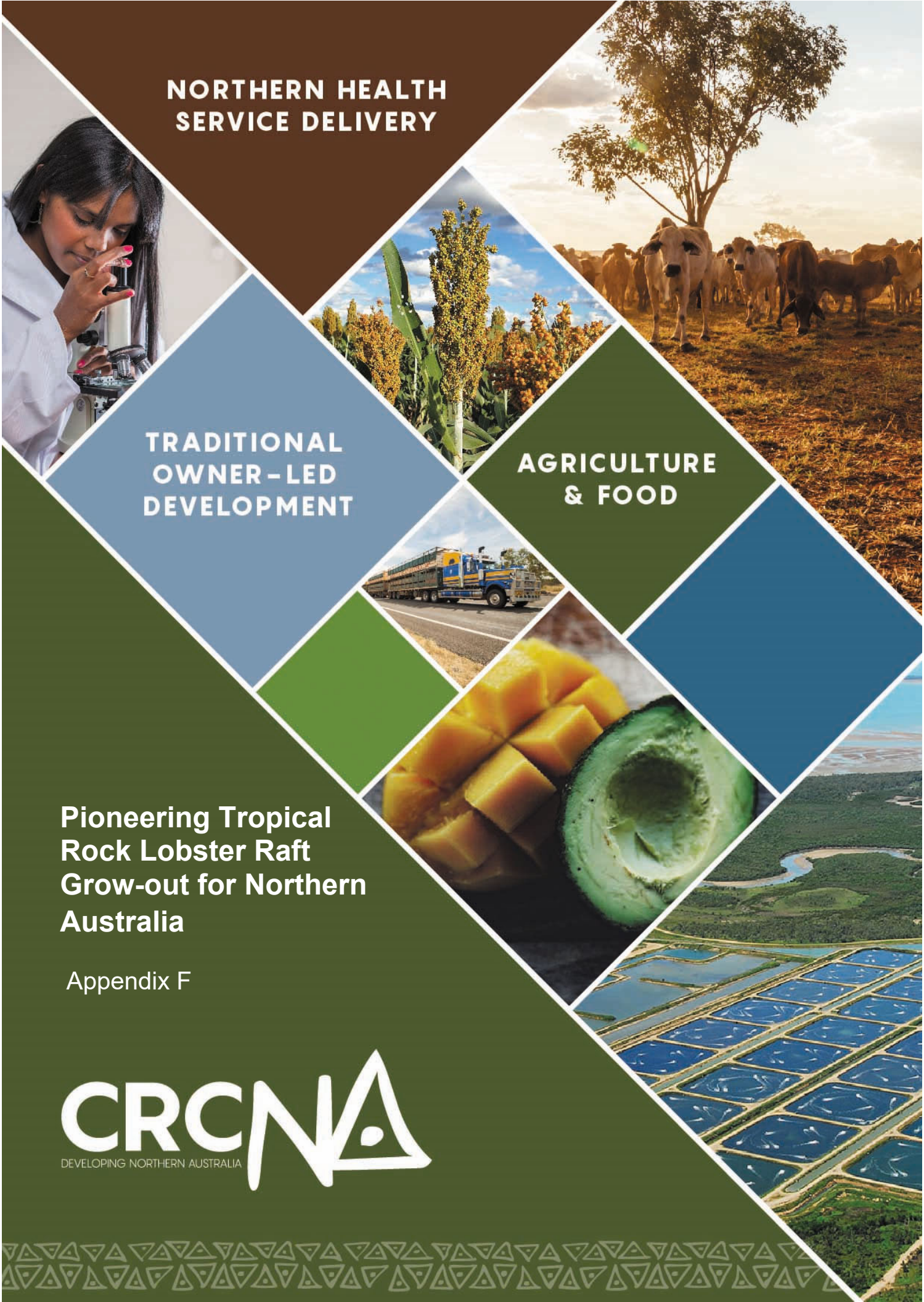
6. WA destination exemption holder requirements

6.1. A batch of TRL juveniles produced at the source hatchery may only be placed into WA waters where the batch of juveniles is certified free of disease (including reportable/notifiable diseases) by the competent authority and is accompanied by the required signed hatchery declaration.

6.2. It is the responsibility of the WA destination exemption holder to comply with the record keeping provisions specified in their exemption to undertake research and development trials.

6.3. After arrival, the destination licence holder must monitor the juveniles and report significant or unusually high levels of mortality, or suspicion of disease, to DPIRD as soon as practicable (and within 24 hours) by calling the WA aquaculture hotline on 1300 278 292.

End of document.



Pioneering Tropical Rock Lobster Raft Grow-out for Northern Australia

Appendix F

CRCNA
DEVELOPING NORTHERN AUSTRALIA

NHEALTH SURVEILLANCE & MANAGEMENT PLAN

1. Introduction

Diseases are one of the major limiting factors for aquaculture development. The occurrence, introduction and spread of pathogens has increased over the years due to the intensification of aquaculture, trade of aquatic animals and their derived products (Rodgers et al., 2011), resistance to antimicrobials and climate change (Bondad-Reantaso et al. 2018; Woo et al., 2020). Challenges posed by exotic, endemic and emerging diseases of aquatic organisms should be tackled in a responsible and efficient manner to ensure the sustainability of the industry (Bondad-Reantaso et al. 2021)

Surveillance and monitoring programs are required for early detection and rapid response to disease outbreaks, early warning of exotic incursions, or emerging diseases. Surveillance is defined as the systematic process of observing and examining samples of population(s) of aquatic animals to detect the presence of infectious agents or occurrence of clinical disease to control disease outbreaks/spread (FAO, 2000). Monitoring refers to all activities directed toward measuring the level of infection or diseases known to be present in a specific population; it involves the systematic collection, analysis and dissemination of information (Cameron, 2002).

Diseases in Tropical Rock Lobsters are understudied worldwide, which is the case for endemic and exotic diseases in QLD and the rest of Australia. The Pathogen Risk Analysis for Aquaculture Biosecurity and Translocation of Tropical Rock Lobsters (*Panulirus ornatus*) in Northern Australia prepared for SeaRaft Research Pty Ltd, identified 39 diseases of potential concern. However, an elimination process of insignificant or irrelevant diseases indicated that two viral, one bacterial, one fungal and three protozoan diseases were of priority and required additional risk mitigation options (Diggles, 2021). These seven disease-causing agents of concern and other non-priority diseases, that can be more common and affect productivity, were considered to develop Ornatas' Health Surveillance and Management Plan (HSMP). The seven disease-causing agents are *Panulirus argus* virus 1 (PaV1), White spot syndrome virus (WSSV), Rickettsia-like organisms (RLO) (bacteria), microsporidians (fungi), haplosporidians, *Hematodinium* spp. and scuticociliates (protozoa).

This HSMP was developed following guidelines for the surveillance of aquatic diseases published by Bondad-Reantaso (2021) and Cameron (2002).

2. Health status of the diseases in Australia and the Ornatas' hatchery

Four of the seven diseases of concern are found in the national, QLD, or WA lists of reportable diseases of aquatic animals, and therefore the causative agents are "under official control". The disease agents are: WSSV (endemic), RLO associated with milky hemolymph disease (exotic), microsporidians, and haplosporidian

parasites. Pathogen-targeted tests will be developed (for endemic disease agents 'under official control') within a JCU- Ornatas Innovation Connections project (pending approval) if a test is not available or has not been validated (explained in more detail below). However, following Diggles' (2021) risk assessment, all agents were considered in this HSMP (gross clinical signs) as they are infectious, expected to cause significant disease, or have been previously detected in QLD.

3. Surveillance objective

Tropical rock lobsters have been held at Ornatas' hatchery in Toomulla, since Aug 2020. Although no formal active surveillance activities have been carried out for any priority or non-priority diseases, mortalities of animals across all life stages (broodstock, phyllosoma, puerulus and juveniles) have been recorded and investigated and there have been no positive cases of any of the diseases of concern.

The HSMP was developed with the understanding that to date there have been no reported cases of the disease-causing agents of concern in Ornatas' facilities and there has been no previous formal active surveillance activity in Ornatas' hatchery and nursery. However, some of the microbial agents listed as under official control could potentially lead to infectious diseases or have been previously detected in QLD. Given this scenario, the objectives of this health HSMP are as follows:

- To investigate the presence or absence of diseases of concern and non-priority diseases in TRL held at Ornatas' hatchery, nursery, and grow-out facilities for every batch of TRL produced
- To secure early detection of any of the diseases of concern or any other unknown or emerging disease
- Set up a transparent reporting system according to national requirements in case of disease detection
- To facilitate translocation of puerulus and juveniles to other jurisdictions and future commercialization while minimising the risk of spreading infectious disease agents
- To demonstrate freedom from each disease of concern (Medium to long term)

4. Target populations

- Individual wild-caught TRL Broodstock entering the hatchery's quarantine system
- Individual batches of phyllosoma produced
- Juveniles of each individual batch produced before translocation to raceways (ponds) or to other jurisdictions
- Potentially market-sized TRL as required. Medium to long-term
- Crustaceans inhabiting Saltwater Creek (shrimps and crabs). Medium to long-term

5. Case definition

Active (proactive) and passive (reactive, if there is a report of disease suspicion) (Bondad-Reantaso, 2021) surveillance will be carried out across the target population (live or moribund) to investigate the presence/ absence of diseases of concern or to early detect new and emerging diseases. Form 1 lists the case definition of diseases of concern and non- priority diseases based on gross clinical signs. This form is to be completed during active and passive surveillance activities in Ornatas' facilities. For some disease-causing agents there are no reliable clinical signs to diagnose infection in TLR (e.g. WSSV), hence the importance of active surveillance and molecular testing. Table 1 shows the sample size per TRL stage and required storage solution to enable

screening for endemic diseases under official control by molecular and histological analyses. A total of 150 animals is the required sample number when the population size is larger than 100,000, in order to detect a pathogen, with a prevalence of 2% (at 95% level of confidence) and assuming that test sensitivity is 100% (Table 2). The corollary is that freedom from disease, based on the same assumptions, can be established with 95% confidence with a sample size of 150.

Table 1. Sample size and storage solution to be used to test for endemic diseases under official control

Test type	Storage	Larvae	Juvenile	Broodstock*
Molecular	80% EtOH	n= 150	n= 150	n= 40
Histology	Davidson	n= 150	n= 150	n= 40

* non-destructive sampling

6. Diagnostic testing

As suggested by FAO, three levels of disease diagnosis will be considered (I, II, and III) (FAO/NACA 2000, 2001)

- Level I: production site observations collected from the following record-keeping documents
  - Hatchery, nursery and broodstock daily data capture
  - Ornatas Aquatic Animal Health Sample Register
  - Passive monitoring reports (gross clinical signs)
  - Water quality data to be collected daily (temperature, salinity, dissolved oxygen and pH) and weekly (ammonia, nitrite, nitrate, phosphate and CO2)
  - Checklists and forms
- Level II: laboratory records of parasitology, bacteriology, mycology and histopathology
  - Weekly Bacterial load across systems and presumptive *Vibrio* (TCBS counts) dynamics
  - Bacterial characterisation
  - Histopathology carried out by AquaPath, Queensland Biosecurity Sciences Laboratory (BSL), or Australian Centre for Disease Preparedness (ACDP) Fish Diseases Laboratory (AFDL)
- Level III: diagnostic testing targeting specialized pathogens or group of pathogens such as virology, electron microscopy, immunology and molecular biology
  - qPCR carried out by AquaPath for targeted bacterial and viral pathogen detection
  - Sequencing for microbiome analysis carried out by Pure Aquatics, UTas or JCU

7. Study design and sampling

Sample collection will be carried out in one day (if practicalities allow) (e.g. 150 late-stage phyllosoma from each batch, or 150 juveniles (if translocation is upcoming), or 40 broodstock with non-destructive sampling) (Figure 1). Staff involved in data/sample collection will move from low (yellow) to high-risk (red) biosecurity areas. Yellow areas represent clean water tanks and larval rearing areas. Yellow areas are the old and new juvenile production systems. The red areas correspond to broodstock and quarantine, artemia production, raceway onshore grow-out, primary filtration and waste channel. Further description biosecurity areas can be found in the Ornatas Biosecurity Plan.

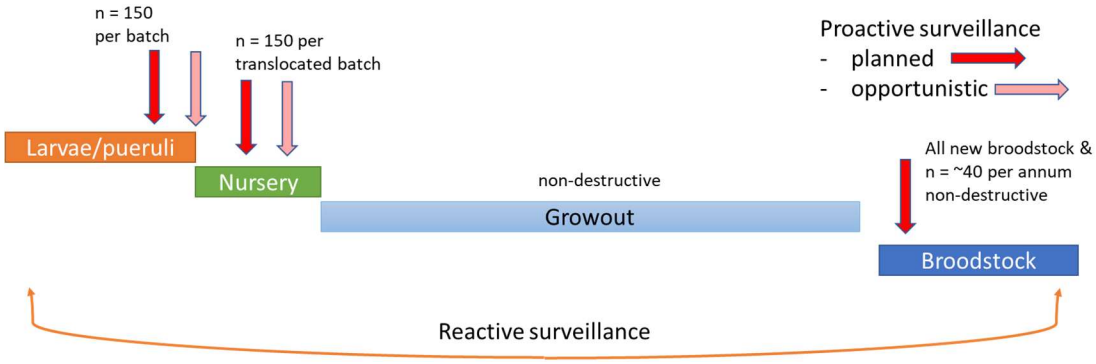


Figure 1. Schematic of disease surveillance sampling events in Tropical Rock Lobsters at Toomulla.

7.1 Larval and juvenile active and passive sampling

For active surveillance purposes, an observational descriptive design will be used, to gather information about the distribution and frequency of a disease per TRL batch, developmental stage, location and time.

Every new batch of high-quality larvae produced will be tested for the permitted four priority diseases of concern at the last phyllosoma stage (stage 11.2 and between 110 and 120 days post-hatching). The population size of the larval batch will be determined from a combination of the number initially stocked (determined from volumetric counts and automated counts from video footage) and the number of larvae removed over time in larval culture. The number of larvae to be tested will be based on population size, assuming a disease prevalence of 2% and perfect test sensitivity as per EpiTools (epitools.ausvet.com.au) sample size calculator for freedom of disease at a 95% level of confidence (Ausvet, 2018; Table 1). An equal number of phyllosoma will be collected from each stocked tank of a RAS system. Depending on size, each larva will be cut in a midsagittal plane to be stored in Davidsons solution (for histology) and at least 80% ethanol (for molecular testing).

Table 1. Sample size required to support freedom of disease assessment with 95% level of confidence based on different population sizes and assuming a disease prevalence of 2%, and test sensitivity of 100%.

Population size	Sample size
50	48
100	78
200	106
300	118
500	130
1000	140
5000	148
10000	149
100,000 +	150

In the case of disease suspicion (passive surveillance required), larvae showing signs of disease will be collected after the daily data capture is carried out (as daily counts include animals showing disease). Culled larvae showing signs of disease will be part of the total number of animals required for batch sampling. If a disease of concern is detected and confirmed, government agencies will be notified as

required and appropriate action taken (e.g. the affected facility immediately isolated, closed and quarantined, while the complete batch will be eliminated and the system disinfected).

In the nursery area, juveniles will be tested at least before translocation to a different jurisdiction. The sample size will be determined by the government agency of the receiving jurisdiction (e.g. for WA calculated based on the total number of animals to be translocated assuming a pathogen prevalence of 2%, a 95% level of confidence and perfect test sensitivity). An equal number of juveniles will be collected from each raceway where the animals from the same batch had been stocked.

TRL younger than juvenile stage 3 (J3) will be cut in a midsagittal plane to be stored in Davidsons solution and 80% ethanol (for molecular testing). Juveniles that have reached the fourth moulting cycle or more (J4>) will be dissected and the organs will be divided into two samples for molecular and histopathology testing. Tissues to be collected are heart, antennal gland, hepatopancreas and hemolymph. Additional samples, such as muscle, gastric mill, gonads, eyes and exoskeleton will be collected, depending on the lesions observed.

In case of evidence of disease in the nursery, prevalence will be estimated, and active sampling will be carried out as previously mentioned. The sample size will be determined based on apparent disease prevalence and professional veterinary advice.

In the case where there is no evidence of disease (no passive sampling undertaken) and no translocation is carried out, opportunistic sampling of up to 150 'old' pueruli that are not required for commercial production will be collected per batch. In the nursery, animals that do not successfully moult (stuck in moult) or have an intermoult period longer than expected (e.g., 25 days from J1 to J2) will be euthanized in an ice slurry and be preserved to be part of the HSMP.

7.2 Broodstock sampling

Every TRL collected from the wild will be tested for the four diseases of concern on transfer to the Ornatas quarantine system and before movement to the broodstock tanks. A sample of hemolymph and/or pleopod from each lobster will be tested for the four diseases of concern. Additionally, each broodstock lobster will be tested with non-destructive methods at least once a year.

Moribund or animals showing an indication of disease will be euthanized in an ice slurry and samples of the internal organs will be obtained for molecular and histopathology purposes. A fraction of each organ will be aseptically dissected and stored in Davidsons solution, 80% ethanol, and DNA/RNA shield or RNA later. Organs to be collected are heart, gonad, antennal gland, hepatopancreas, gastric mill and hemolymph or any tissue that is visually detected as abnormal. The rest of the animal will be stored at -20 °C.

8. Validation and quality assurance

- Feedback from internal (SC) and external evaluation
- Pilot survey to be carried out
- Audit and corrective measures to be taken

9. Sample submission and aquatic pathology laboratories

Samples collected as a result of passive surveillance when there is evidence of high mortality and/or suspicion of a disease of concern will be submitted to the Australian Centre for Disease Preparedness (ACDP) (formerly known as the Australian Animal Health Laboratory - AAHL) - Fish Diseases Laboratory (AFDL) via the Queensland government's Biosecurity Sciences Laboratory (BSL). Samples collected from active surveillance will be submitted to AquaPath laboratory at James Cook University. As there is only one test accredited by the National Association of Testing Authorities (NATA) (for WSSV in shrimps), AquaPath will develop destructive and non-destructive molecular based pathogen detection assays for broodstock, hatchery, nursery and grow-out stages through an Innovation Connections Grant. This project will also focus on the development of treatments, especially antibiotic alternatives and the production of a histopathology atlas.

If a notifiable disease (e.g. WSSV) is detected and confirmed, the company will adhere to the required reporting to government agencies (i.e. Biosecurity Queensland) and will work with government agencies on required actions (e.g. possible elimination of TRL in the entire system (where other TRL of the same batch were at risk of infection). A health monitoring and management plan will be developed for each detected notifiable disease.

Emergency Preparedness and contingency plan

Preparedness is crucial for ensuring the effectiveness of emergency responses. Australia has established contingency planning measures for addressing outbreaks of aquatic animal diseases, which include the utilization of the Australian Aquatic Veterinary Emergency Plan (AQUAVETPLAN) manuals. Crustacean diseases included in The AQUAVETPLAN are Crayfish Plague and White Spot Disease (WSD). These manuals outline the control principles to be employed in response to a suspected or confirmed incursion of the disease in Australia. While Crayfish Plague is a freshwater fungal disease, as mentioned in Diggles' Risk Assessment (2010), White Spot Disease (WSD) is a viral disease that affects all decapod crustaceans. The manual from the Department of Agriculture (2013) provides a description of the principles of control and eradication, as well as the preferred response options in Australia, including eradication, containment, control and zoning, and control and disease mitigation. Details of Ornatas' emergency preparedness and contingency are included in the Ornatas Biosecurity Management Plan.

References

- Ausvet. (2018). Publications and Resources. EpiTools. https://www.ausvet.com.au/tools-resources/

- Baldock, F. C., Blazer, V., Callinan, R., Hatai, K., Karunasagar, I., Mohan, C. V., ... & Bondad-Reantaso, M. G. (2005). Outcomes of a short expert consultation on epizootic ulcerative syndrome (EUS): Re-examination of causal factors, case definition and nomenclature. In P. Walker, R. Lester, & M. G. Bondad-Reantaso (Eds.), Diseases in Asian Aquaculture V (pp. 555–585). Fish Health Section, Asian Fisheries Society.

- Bondad-Reantaso, M. G., Garrido-Gamarro, E., & McGladdery, S. E. (2018). Climate change-driven hazards on food safety and aquatic animal health. FAO Fisheries and Aquaculture Technical Paper No. 627, 517–534. FAO.

- Bondad-Reantaso, M. G., Fejzic, N., MacKinnon, B., Huchzermeyer, D., Seric-Haracic, S., Mardones, F. O., ... & Dall'occo, A. (2021). A 12-point checklist for surveillance of diseases of aquatic organisms: A novel approach to assist multidisciplinary teams in developing countries. Reviews in Aquaculture, 13, 1469-1487. https://doi.org/10.1111/raq.12530

- Cameron, A. (2002). Survey Toolbox for Aquatic Animal Diseases: A Practical Manual and Software Package. ACIAR Monograph.

- Department of Agriculture. (2013). Disease strategy: White spot disease (Version 2.0). In Australian Aquatic Veterinary Emergency Plan (AQUAVETPLAN). Australian Government Department of Agriculture.

- Diggles, B. (2021). Pathogen risk analysis for aquaculture biosecurity and translocation of tropical rock lobsters (Panulirus ornatus) in northern Australia. DigFish Services report DF 21-03b.

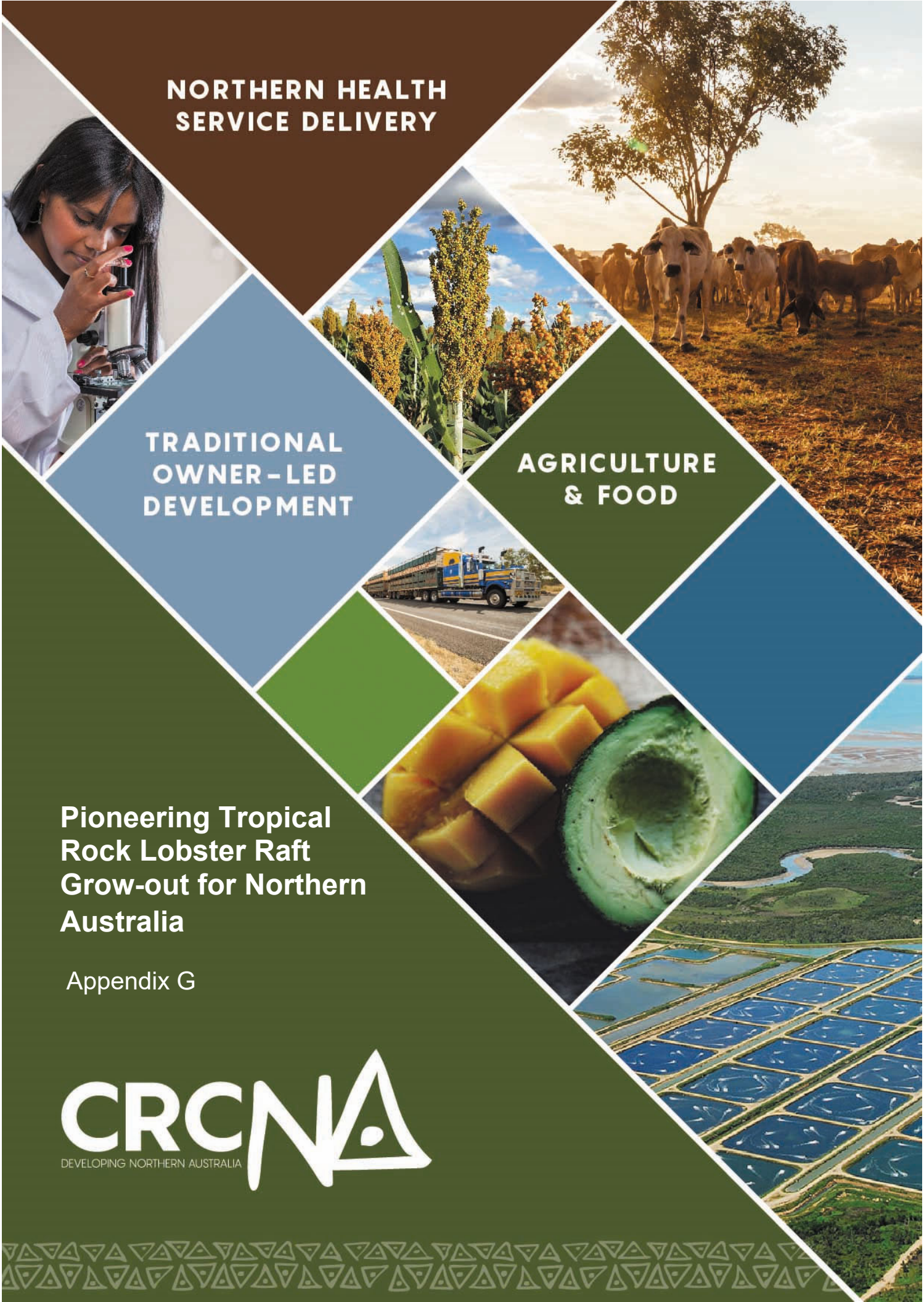
- Diggles, B. K., & Arthur, J. R. (2010). Pathogen risk analysis for aquatic animals: Experiences from nine case studies. In M. G. Bondad-Reantaso, J. B. Jones, F. Corsin, & T. Aoki (Eds.), Diseases in Asian Aquaculture VII (pp. 271-290). Fish Health Section, Asian Fisheries Society.

- Food and Agriculture Organization (FAO). (2007). Aquaculture development: Health management for responsible movement of live aquatic animals. FAO Technical Guidelines for Responsible Fisheries. No. 5, Suppl. 2.

- Food and Agriculture Organization/Network of Aquaculture Centres in Asia-Pacific (FAO/NACA). (2000). Asia Regional Technical Guidelines on Health Management for the Responsible Movement of Live Aquatic Animals and the Beijing Consensus and Implementation Strategy. FAO Fisheries Technical Paper No. 402.

- World Organisation for Animal Health (OIE). (2021). Aquatic Animal Health Code: Chapter 2.1 Import Risk Analysis. OIE.

- Woo, P. T. K., Leong, J.-A., & Buchmann, K. (Eds.). (2020). Climate Change and Infectious Fish Diseases. CABI.



Pioneering Tropical Rock Lobster Raft Grow-out for Northern Australia

Appendix G

CRCNA
DEVELOPING NORTHERN AUSTRALIA

Field Day Report

CRCNA: Pioneering Tropical Rock Lobster Raft Grow-out for Northern Australia

Project number: A.3.2021116

Date: 14 April 2024

Introduction

One of the key research activities of the project *Pioneering Tropical Rock Lobster Raft Grow-out for Northern Australia* was the dissemination of research findings through a Field Day event. This five-hour event was hosted at the Ornatas Aquaculture Facility in Toomulla, QLD on Wednesday 13th March 2024. During the Field Day, the Ornatas Management team and the Project Manager presented an overview of Ornatas as a company and its progress since it was established in 2018. The development of the hatchery and nursery facilities and their major achievements and challenges were shared with the attendees. The Project, which encompassed the next production stage, the grow-out of hatchery-produced juvenile lobsters, was thoroughly explained by breaking down its six work packages. The project results, business model, challenges, opportunities and research needs to develop the Tropical Rock Lobster Aquaculture Industry in Northern Australia, were discussed with the attendees and are included in the Project final report.

Objectives

The main goal of the Field Day was to disseminate knowledge gained by Ornatas throughout the life of the Project on the development, current status and production models of the TRL aquaculture industry. The intention was to have an open discussion with aquaculture experts from industry and universities and with entities that promote economic development, innovation, and sustainability in Northern Australia to evaluate the outcomes of the project, research needs and opportunities to develop the new TRL Aquaculture industry in NA.

Field Day Schedule

8:30-9:00 - Participant arrival and sign-in at Ornatas

Presentations in Ornatas' new meeting room (copy of presentations in Appendix E)

9:00 – Welcome and introductions

9:05 – CRCNA welcome

9:10 – FRDC welcome

9:15 – 9:30 – Ornatas company progress

9:30 – 9:45 – The SeaRaft Project

9:45 – 10:00 – Virtual tour of the nursery

10:00 – 10:15 – SeaRaft project activities and findings

10:15 – 10:25 – Business model

10:25 – 10:45 – Discussion of Tropical Rock Lobster aquaculture in Northern Australia – what next?

10:45 – 11:15 - Morning tea, discussion with Ornatas team and Display/demonstration of Tropical Rock Lobster life stages – larvae to broodstock

11:15 – 12:15 – Physical tour of the grow-out facility - ponds and raft grow-out systems

12:30 – 13:30 – Lunch, informal discussion and end of event

Participants

The 20 Field Day attendees represented a diverse group that overall, work towards the sustainable development and prosperity of Northern Australia through research, innovation, investment, collaboration, and policy coordination. The table below (Table 1) shows the list of attendees from the Cooperative Research Centre for Northern Australia (CRCNA), the Fisheries Research and Development Corporation (FRDC), The Office of Northern Australia, AusIndustry, two Australian Universities (James Cook Universities and University of Tasmania), the Ornatas management team, and the Project Steering Committee members from Maxima and Ornatas. Apologies were sent from other members of the Aquaculture Industry sector, including the prawn and barramundi sectors, and other project Partners (specifically, Honey & Fox and JSJ Seafood).

Table 1. List of attendees to the End of project Field Day

Name	Organisation	Role
Sarah Docherty	CRCNA	CEO_Senior Project Manager
Anthony Curro	CRCNA	CEO_Senior Project Manager
Sheriden Morris	CRCNA	Board
John Wharton	CRCNA	Board
Rebecca Mohr-Bell	CRCNA	Board
Peter Long	CRCNA	Board
Josh Fielding	FRDC	Senior Research Portfolio Manager
Kylie Dunstan	FRDC	General Manager Stakeholder Engagement
John Chandler	Aus Industry	Regional Manager
Dean Jerry	JCU CSTFA	Professor. Director of ARC Research Hub
Kyall Zenger	JCU CSTFA	Professor
Gregory Smith	IMAS	Director ARC Research Hub for Sustainable Lobster Aquaculture
Basseer Codabaccus	IMAS	Senior Research Fellow ARC
Sally Butler	Office of Northern Australia	Assistant Director
Frances Kaczmarek	Office of Northern Australia	Partnerships and Projects Division
Jennifer Blair	Ornatas_Project SC	Hatchery and R&D Manager
Sandra Infante Villamil	Ornatas_Project SC	Project Manager and R&D Officer
Tony Barton	Ornatas	Farm Manager
John Breen	Ornatas	Grow-out and nursery Manager
Steven Gill	Maxima_Project SC	General Manager

Field Day activities and comments from participants

Participants arrive at Ornatas aquaculture facility at Toomulla Beach in QLD

Tony Barton, Ornatas General Manager, explaining the Company's progress at Ornatas' new meeting room

Participants discussing the Project outcomes and evaluating opportunities for the development of the Tropical Rock Lobster aquaculture industry in Northern Australia

Attendees on the raft system used to evaluate grow-out of hatchery produced TRL at Ornatas aquaculture facility at Toomulla Beach in QLD

Thank you again for a fabulous, informative and fun day. Your facility is impressive and your staff are a credit to you. Congratulations on success a successful field day.

Best wishes

Kylie

Field Day communication

Ornatas Pty Ltd
2,541 followers
1d •

We're coming close to the end of a research era, as we recently held a final technical Field Day for the CRCNA Pioneering Tropical Rock Lobster Raft Grow-out project. The project aimed to investigate Tropical Rock Lobster sea raft grow out systems in Northern Australia, and it certainly has not been without its challenges, since the project launch in 2021.

We've worked hard with our research and industry partners to build knowledge, modify systems in two NA locations, and develop innovative technology and operations that are resilient in unpredictable conditions, and to understand sometimes unpredictable markets! We've persevered though, as we do, and our focus areas encompassed the environment for growing lobsters, raft design, lobster health and translocation protocols, feeding strategies, growth performance, and market acceptability. Pushing some new boundaries in aquaculture innovation.

This Field Day welcomed many of the @Cooperative Research Centre for Developing Northern Australia Board, senior managers from FRDC (Kylie Dunstan, Josh Fielding), UTas (Greg Smith and Basseer Codabaccus) and Maxima project partners, Office of Northern Australia delegates, AusIndustry, and JCU scientists (Dean Jerry and Kyall Zenger). The event included:

🌀 A display/demonstration of Tropical Rock Lobster life stages – larvae to broodstock
👀 A virtual tour of the nursery
🦞 A physical tour of the grow-out facility
💬 A discussion on the future of Tropical Rock Lobster aquaculture in Northern Australia

It was a great day sharing the accomplishments and learnings we've made through undertaking this research project!

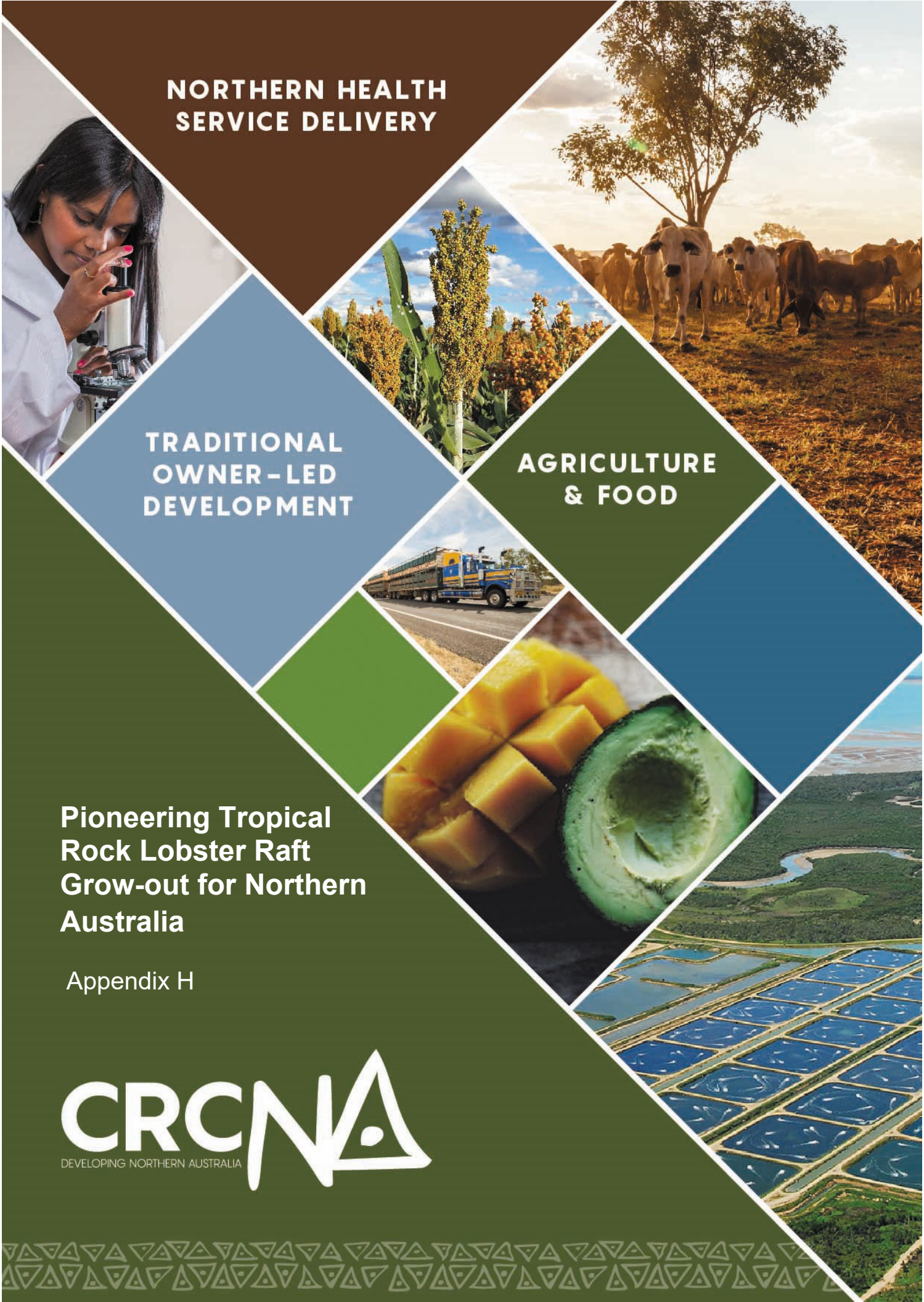
A big thank you to those who attended the Field Day, to the Ornatas team for setting up displays and talking to our guests about our amazing lobsters, and to the following partners who helped make this project possible:

Scott Parkinson Tony Barton Jennifer Blair John Breen Sandra Infante Villamil Maxima The Opportunity Group PFG Group JSJ Seafood Cooperative Research Centre for Developing Northern Australia (CRCNA) FRDC - Fisheries Research and Development Corporation Honey & Fox @institute for marine and antartic studies

#ornatas #lobsteraquaculture #sustainableseafood #tropicalrocklobsters

Link

https://www.linkedin.com/posts/ornatas_ornatas-lobsteraquaculture-sustainableseafood-activity-7181411361522937858-q-me?utm_source=share&utm_medium=member_desktop



Pioneering Tropical Rock Lobster Raft Grow-out for Northern Australia

Appendix H

CRCNA
DEVELOPING NORTHERN AUSTRALIA

Pioneering Tropical Rock Lobster Raft Grow-out for Northern Australia

ornatas

Field Day

1

Welcome

CRCNA

FRDC
FISHERIES RESEARCH AND DEVELOPMENT CORPORATION

2

ornatas

Company progress

Tony Barton – General Manager

3

ornatas

The SeaRaft Project

Jennifer Blair – Hatchery and R&D Manager

4

The hatchery and nursery virtual tour

Jennifer Blair – Hatchery and R&D Manager

John Breen – Nursery and Grow-out Manager

5

SeaRaft project activities and findings

Sandra Infante Villamil – Project Manager and R&D officer

6

Business model

7

Discussion

Tropical Rock Lobster aquaculture in Northern Australia – what next?

8

Commercialising
Tropical Rock Lobster Aquaculture
in Northern Australia

ornatas

9

Passionate People

- Board and investors
- Ornatas' workforce
- Local contractors
- Research partners
- Government support
- Funding bodies
- Industry partners
- Local community
- Aquaculture community

10

20 years in the making...

Ornatas is commercialising world's first science (UTas' IMAS division)

Fully operational **Tropical Rock Lobster Hatchery**

Infrastructure investment funded by Ornatas, plus a Northern Australia Business Development Grant for expansion projects.

The future:
Ornatas is working with its partners to bring Tropical Rock Lobster grow-out to Northern Australia in onshore aquaculture systems, to create a **premium, sustainable, Australian Tropical Rock Lobster product.**

11

Our Strategy

Ornatas' Vision

To improve **Australia's Tropical Rock Lobster industry** for domestic and international markets

Operational Developments

Northern Australia:
Onshore Lobster Aquaculture (Toomulla Beach site)

Fully renovated facility

Current target production reached in ~4 years

Commercially operating hatchery and Growout
=
50 tonnes of Tropical Rock Lobsters In Onshore Culture

10-year Australian Industry growth plan

Developing Australia's Tropical Rock Lobster industry by adding >**$100 million** (revenue) **plus** employment for QLD and Northern Australia

12

Major Achievements

- 268 ha freehold
- 30 staff
- Commercial hatchery (90,000 J1s p.a. capacity)
- Facilities upgrade
- Grow-out development
- Risk mitigation (water storage)
- Solar system and car park
- NABD Grant

13

Tropical Rock Lobster
Panulirus ornatus

Phyllosoma *Puerulus*

Technically challenging and sensitive larval stages

Grown in state-of-the-art hatchery

Land-based nursery systems and grow out using high-end and sustainable systems

Optimum feed and water quality

24-month cycles from egg to market

Adult

14

Pioneering Tropical Rock Lobster Raft Culture for Northern Australia

ornatas

15

Research Project

WP1: Assess and monitor farm site environment

WP2: Design and evaluate sea raft systems

WP3: Develop and test juvenile translocation

WP4: Assess and refine feeding strategy

WP5: Test stocking density and biofouling management

WP6: Evaluate product quality for a diversified market

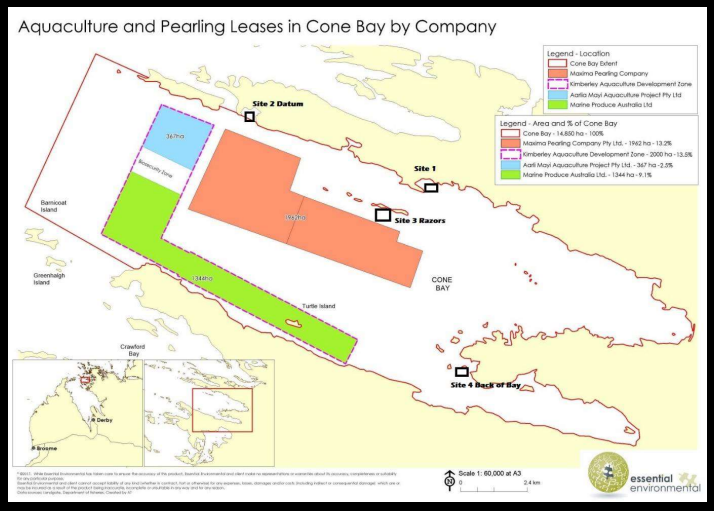
Addressing challenges in the production cycle through interdisciplinary research to produce market ready premium lobster and to build a sustainable industry

CRCNA FRDC ornatas

16

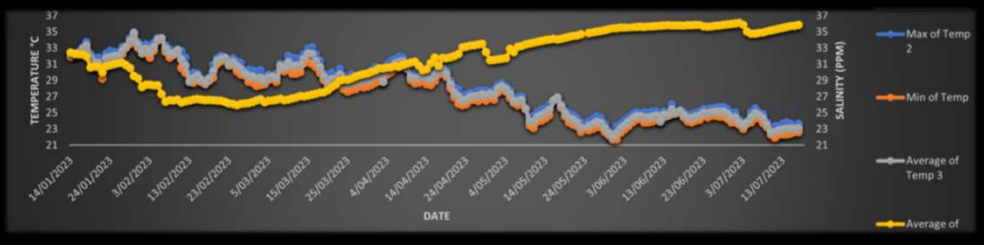
Environmental Management and Monitoring

WP1: Assess and monitor farm site environment



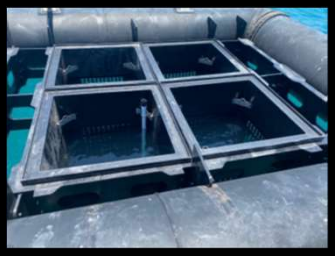
17

WP1: Assess and monitor farm site environment



18

WP2: Design and evaluate sea raft systems



maxima

19

WP2: Design and evaluate sea raft systems

20

WP3: Develop and test juvenile translocation

Department of Primary Industries and Regional Development

DPIRD assessment of disease risks regarding a proposal to import tropical rock lobster juveniles from a Queensland hatchery for grow out in Western Australian waters

FINAL CONSULTATION VERSION

12 April 2022

PATHOGEN RISK ANALYSIS FOR AQUACULTURE BIOSECURITY AND TRANSLOCATION OF TROPICAL ROCK LOBSTERS (*Panulirus ornatus*) IN NORTHERN AUSTRALIA

Prepared by:

Ben Diggles PhD

DigsFish Services Pty Ltd, Banksia Beach, QLD 4507

Prepared for:

SeaRaft Research Pty Ltd,
PO Box 774 Deeragun, QLD 4818

Version Control:

First Draft Hazard ID	27 April 2021
Draft Risk Analysis	20 October 2021
Final Version	29 November 2021

DIGSFISH SERVICES

Disclaimer

This document was prepared as an independent pathogen risk assessment. The views expressed are those of the author. DigsFish Services does not warrant that the information in this document is free from errors or omissions. The authors do not accept any form of liability, be it contractual, tortious or otherwise, for the contents of this document or for any consequences arising from its use or any reliance placed upon it.

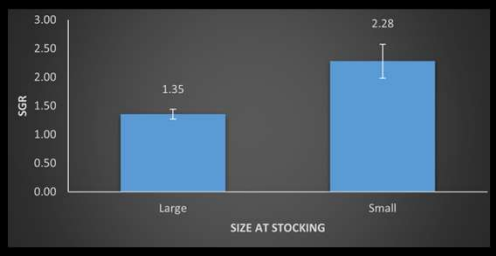
2

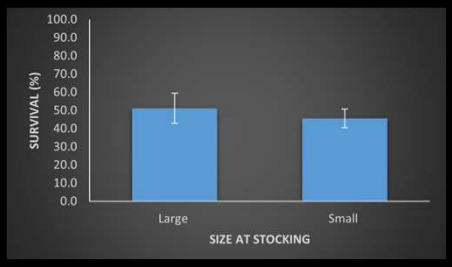
21

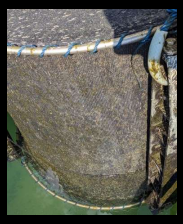
WP4: Assess and refine feeding strategy

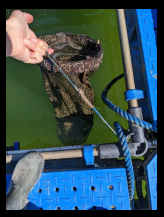
22

WP5: Test stocking density and biofouling management









23

WP6: Evaluate product quality for a diversified market

24

Supplying market-ready premium lobster product and to build a sustainable TRL industry

25

The Sea Raft Project:

Under a collaborative project funded by the CRCNA and supported by FRDC, our aim is to bring Tropical Rock Lobster sea raft grow-out to Northern Australia

Project partners: maxima, Three Capes Marketing, Honey & Fox, PFG Group, University of Tasmania, IMAS, ornatas

Funded by: FRDC, CRCNA

26

Thank you

27

Commercialising
Tropical Rock Lobster Aquaculture
in Northern Australia

ornatas

1

Passionate People

- Board and investors
- Ornatas' workforce
- Local contractors
- Research partners
- Government support
- Funding bodies
- Industry partners
- Local community
- Aquaculture community

2

20 years in the making...

Ornatas is commercialising world's first science (UTas' IMAS division)

Fully operational **Tropical Rock Lobster Hatchery**

Infrastructure investment funded by Ornatas, plus a Northern Australia Business Development Grant for expansion projects.

The future:
Ornatas is working with its partners to bring Tropical Rock Lobster grow-out to Northern Australia in onshore aquaculture systems, to create a **premium, sustainable, Australian Tropical Rock Lobster product.**

3

Our Strategy

Ornatas' Vision

To improve **Australia's Tropical Rock Lobster industry** for domestic and international markets

Operational Developments

Northern Australia:
Onshore Lobster Aquaculture (Toomulla Beach site)

Fully renovated facility

Current target production reached in ~4 years

Commercially operating hatchery and Growout
=
50 tonnes of Tropical Rock Lobsters In Onshore Culture

10-year Australian Industry growth plan

Developing Australia's Tropical Rock Lobster industry by adding >**$100 million** (revenue) **plus** employment for QLD and Northern Australia

4

Major Achievements

- 268 ha freehold
- 30 staff
- Commercial hatchery (90,000 J1s p.a. capacity)
- Facilities upgrade
- Grow-out development
- Risk mitigation (water storage)
- Solar system and car park
- NABD Grant

5

Tropical Rock Lobster
Panulirus ornatus

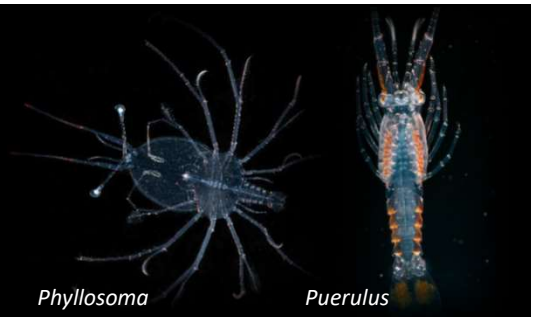
Technically challenging and sensitive larval stages

Grown in state-of-the-art hatchery

Land-based nursery systems and grow out using high-end and sustainable systems

Optimum feed and water quality

24-month cycles from egg to market



Phyllosoma *Puerulus*

Adult

6

Grow-out

- 2 Trial ponds (0.1 ha)
- Several stocking events
- Different seasons
- Different sized juveniles
- Stocking density
- Refining feeding technology
- Refining operations and system design

7

Pioneering Tropical Rock Lobster Raft Culture for Northern Australia

ornatas

1

Research Project

WP1: Assess and monitor farm site environment

WP2: Design and evaluate sea raft systems

WP3: Develop and test juvenile translocation

WP4: Assess and refine feeding strategy

WP5: Test stocking density and biofouling management

WP6: Evaluate product quality for a diversified market

Addressing challenges in the production cycle through interdisciplinary research to produce market ready premium lobster and to build a sustainable industry

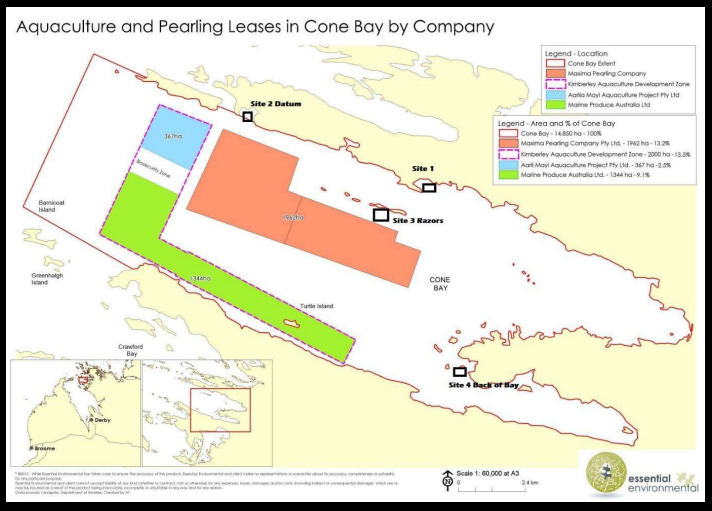
CRCNA FRDC ornatas

2

Environmental Management and Monitoring

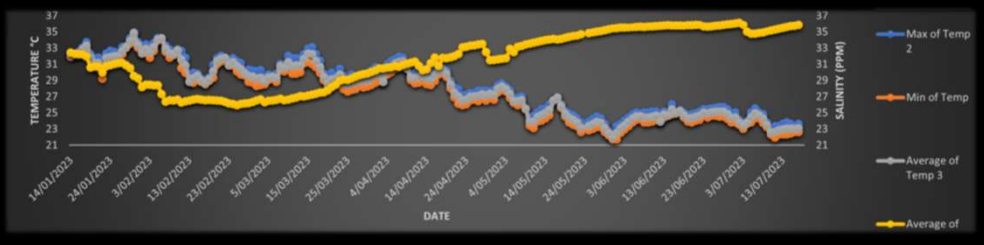
WP1: Assess and monitor farm site environment

maxima
the opportunity group



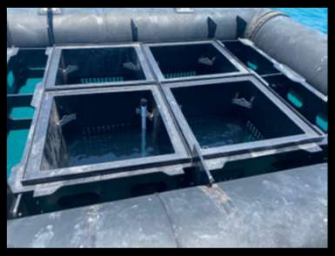
3

WP1: Assess and monitor farm site environment



4

WP2: Design and evaluate sea raft systems



maxima

5

WP2: Design and evaluate sea raft systems

6

WP3: Develop and test juvenile translocation

Department of Primary Industries and Regional Development

DPIRD assessment of disease risks regarding a proposal to import tropical rock lobster juveniles from a Queensland hatchery for grow out in Western Australian waters

FINAL CONSULTATION VERSION

12 April 2022

PATHOGEN RISK ANALYSIS FOR AQUACULTURE BIOSECURITY AND TRANSLOCATION OF TROPICAL ROCK LOBSTERS (*Panulirus ornatus*) IN NORTHERN AUSTRALIA

Prepared by:

Ben Diggles PhD

DigsFish Services Pty Ltd, Banksia Beach, QLD 4507

Prepared for:

SeaRaft Research Pty Ltd,
PO Box 774 Deeragun, QLD 4818

Version Control:

First Draft Hazard ID	27 April 2021
Draft Risk Analysis	20 October 2021
Final Version	29 November 2021

DIGSFISH SERVICES

Disclaimer

This document was prepared as an independent pathogen risk assessment. The views expressed are those of the author. DigsFish Services does not warrant that the information in this document is free from errors or omissions. The authors do not accept any form of liability, be it contractual, tortious or otherwise, for the contents of this document or for any consequences arising from its use or any reliance placed upon it.

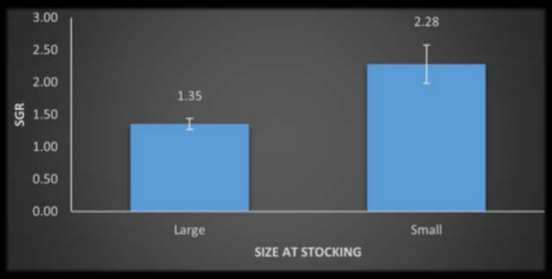
2

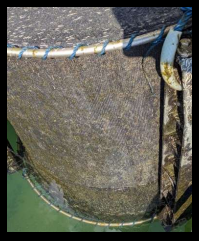
7

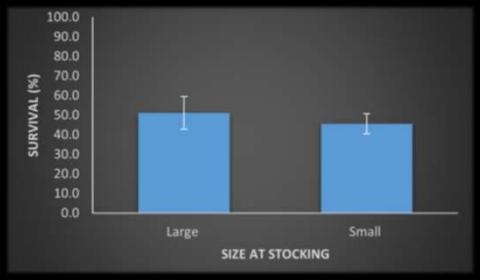
WP4: Assess and refine feeding strategy

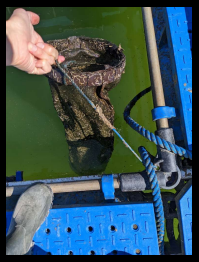
8

WP5: Test stocking density and biofouling management









9

WP6: Evaluate product quality for a diversified market

10

Supplying market-ready premium lobster product and to build a sustainable TRL industry

11

The Sea Raft Project:

ornatas

Under a collaborative project funded by the CRCNA and supported by FRDC, our aim is to bring Tropical Rock Lobster sea raft grow-out to Northern Australia

Project partners: maxima the opportunity group; Three Capes Marketing by JSJ Seafood; Honey & Fox; PFG Group; University of Tasmania; IMAS Institute for Marine & Antarctic Studies

Funded by: FRDC Fisheries Research & Development Corporation; CRCNA

12

Thank you

13

Hatchery

1

2

Hatchery update

- 4 broodstock populations
- Repeat spawns per season
- Up to 1.4 million hatched larvae per clutch
- Year-round egg supply

3

Hatchery update

- Consecutive batches of pueruli and juveniles
- >50,000 pueruli in 13 batches
- 25,000 juveniles
- Increased experience of technical staff with technology, managing water quality, and larval husbandry
- Productivity variable among batches – continuing to learn more

4

5

Nursery update

- New nursery facility operational Feb 23
- Capacity of 90,000 juveniles p.a.
- Automated feeding

Key challenges

- Cannibalism
- Feeds and feeding strategy

6

7

Grow-out update

8

Grow-out

- 2 Trial ponds (0.1 ha)
- Several stocking events
- Different seasons
- Different sized juveniles
- Stocking density
- Refining feeding technology
- Refining operations and system design

9

Pioneering Tropical Rock Lobster Raft Culture for Northern Australia. Activities and findings



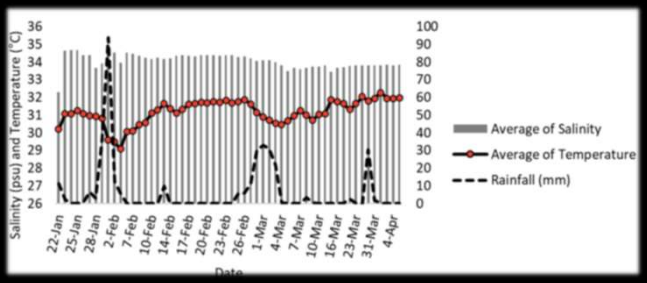

1

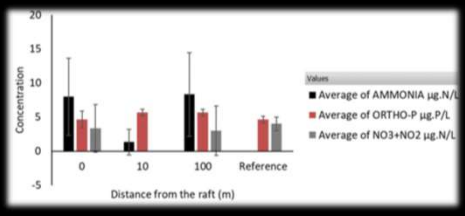
Environmental Management and Monitoring

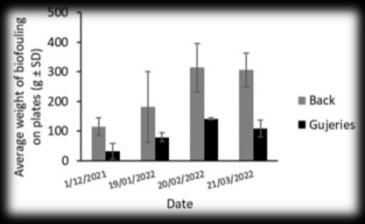
WP1: Assess and monitor farm site environment

maxima
the opportunity group

Site selection
Baseline information







2

WP1: Assess and monitor farm site environment

Design, implement and refine environmental monitoring and management plan (EMMP) for land-based lobster grow-out

General objective

- Examine the impact of land-based raft production on water quality
- Examine the effect of the environment on pond productivity

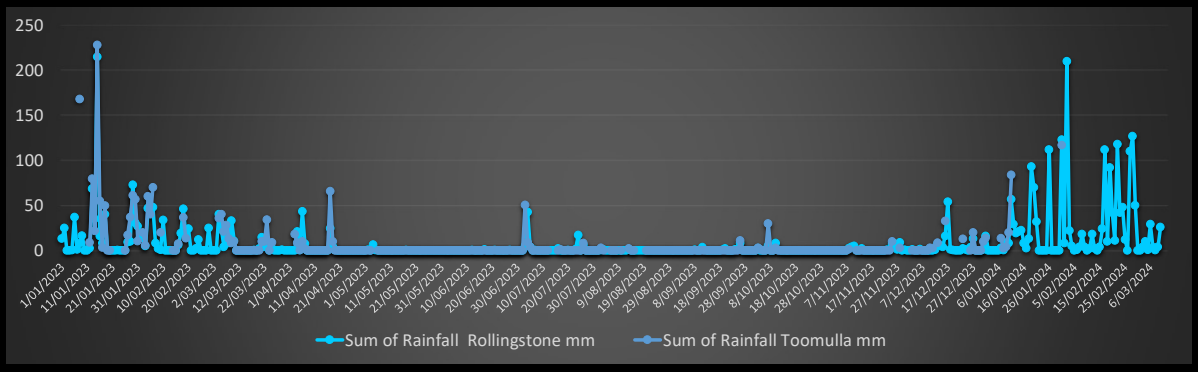
3

4

Rainfall (mm)

2024	1003.1
Jan	416.5
Feb	547.8
Mar	38.8

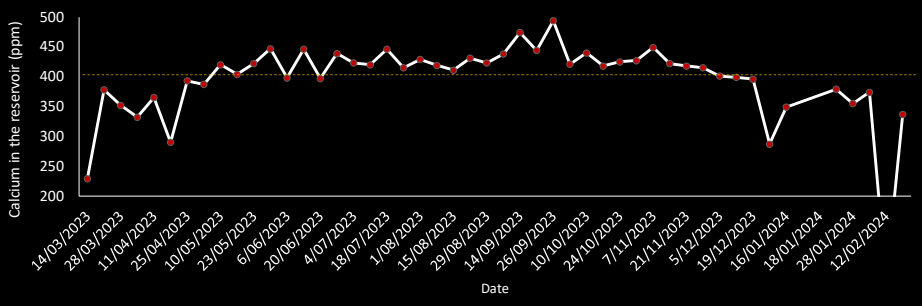
2023 1564.2



5

Effect of the environment on pond productivity

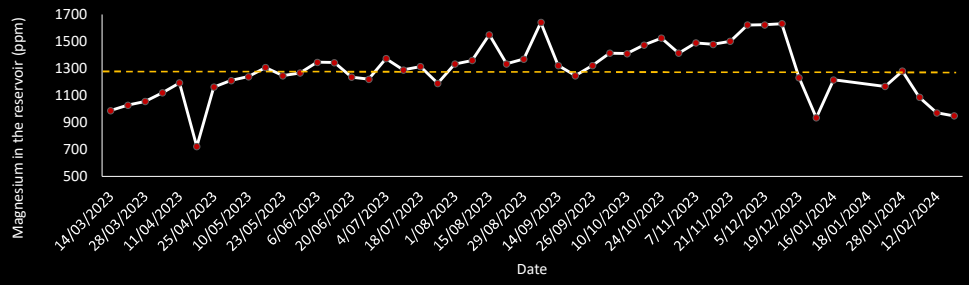
Reservoir ions. Calcium



6

The environment on pond productivity.

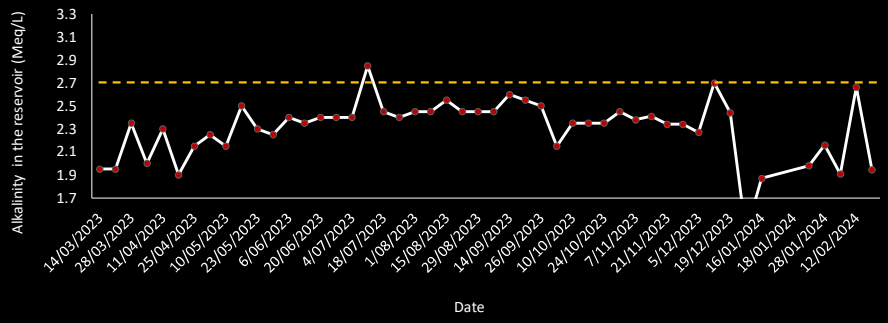
Reservoir ions. Magnesium



7

The environment on pond productivity.

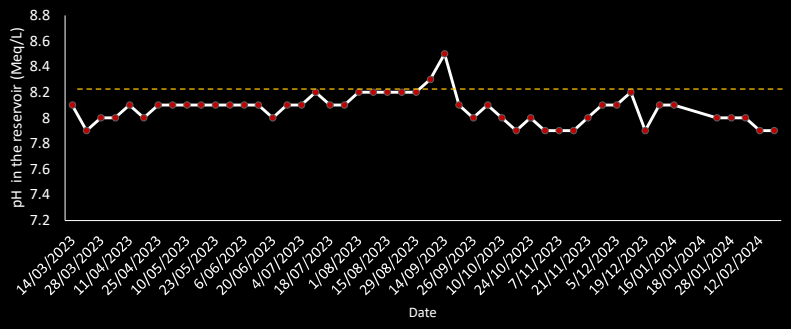
Alkalinity



8

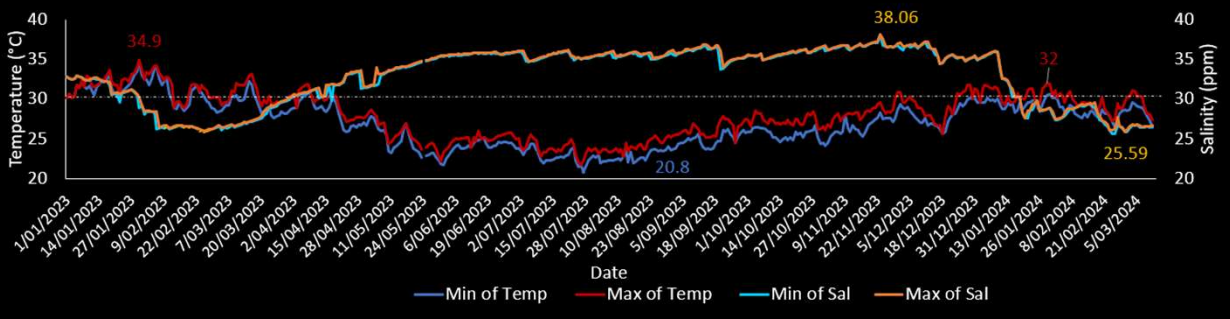
The environment on pond productivity.

pH



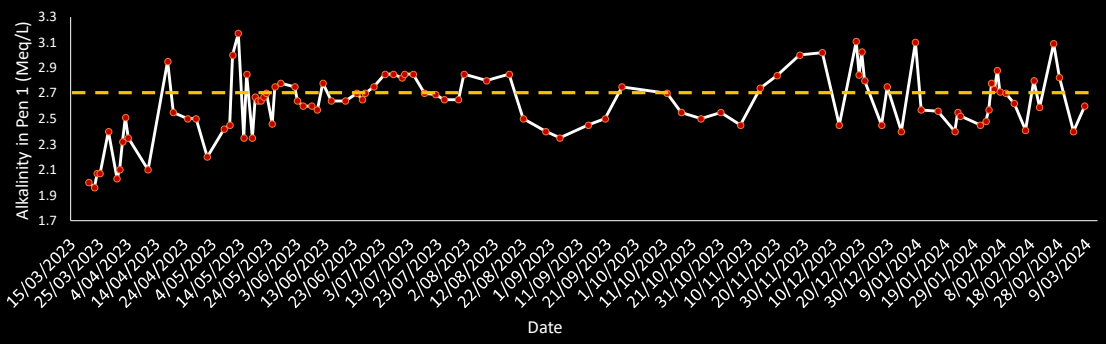
9

Temperature (°C) and salinity (ppm)



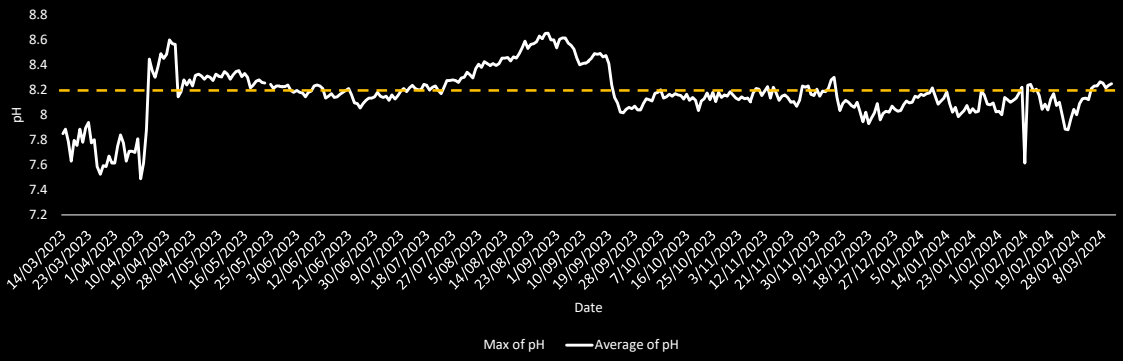
10

Alkalinity



11

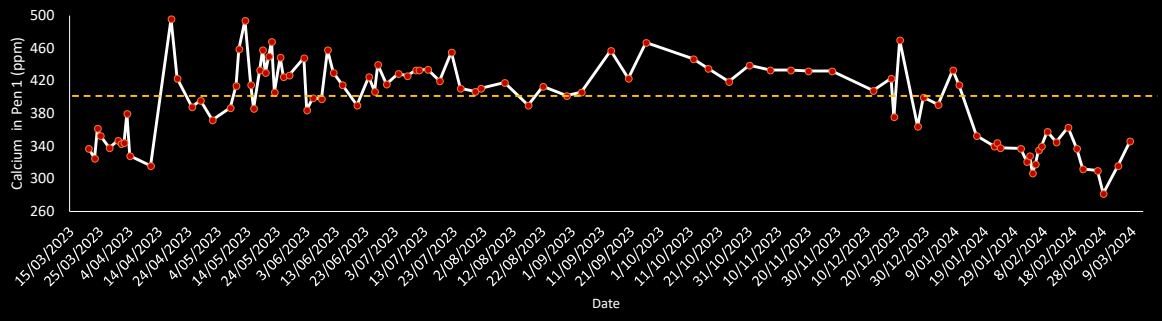
pH



WP 1

12

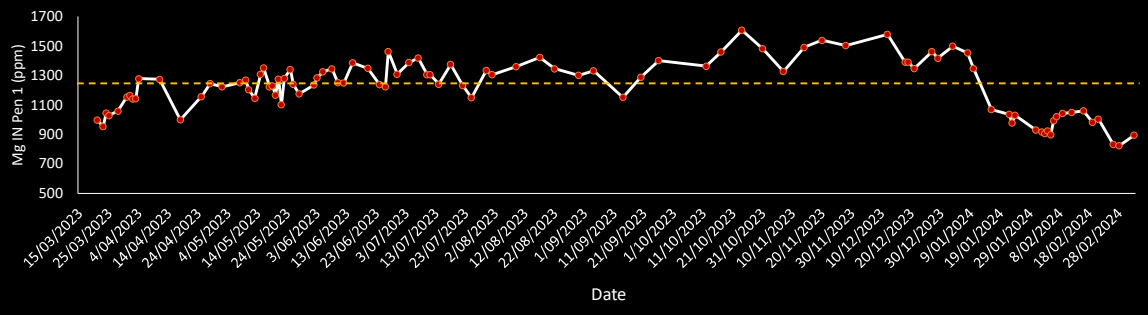
Calcium



WP 1

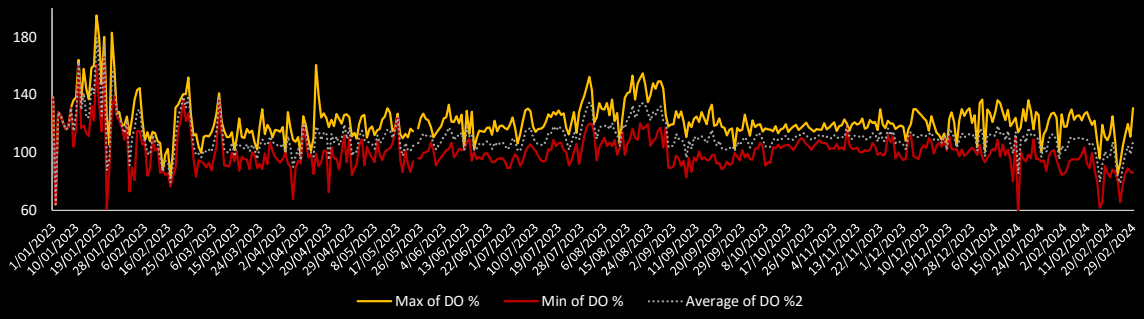
13

Magnesium



14

Dissolved Oxygen (DO)

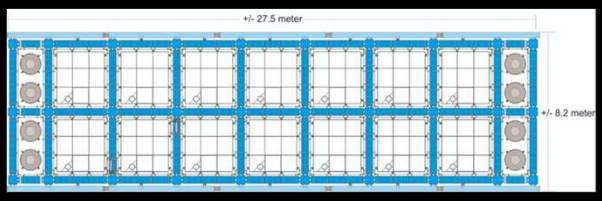


WP 1

15

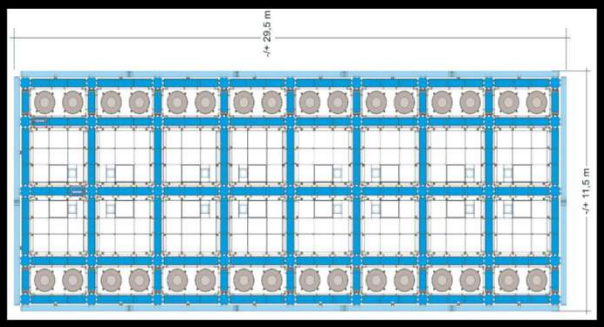
WP2: Design and evaluate sea raft systems

16



Raft 1. Aquatec frame design

17



Raft 2

18

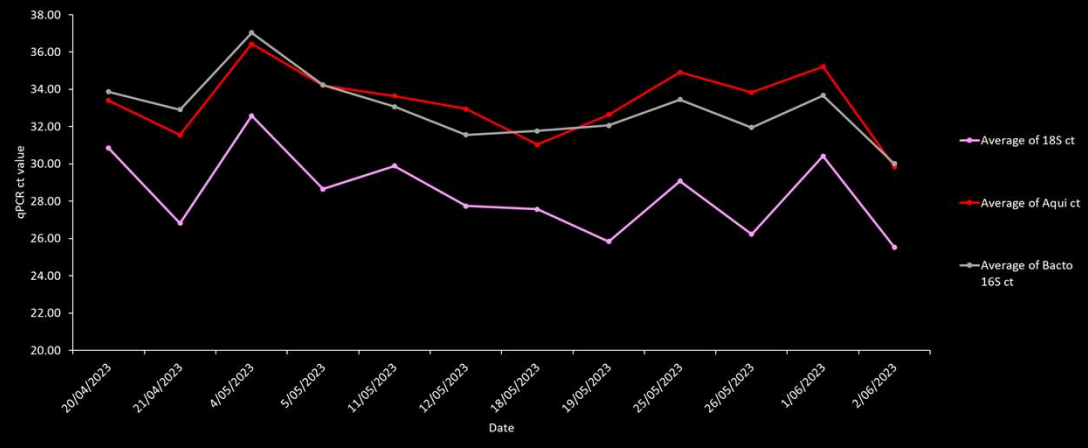
WP3: Develop and test juvenile translocation

AAH
Aquatic animal health

HSMP
- **Active**
- **Passive. AquaPath and or BSL**

19

eDNA pilot project



20

WP4: Assess and refine feeding strategy

21

WP4: Assess and refine feeding strategy

Feeding management approach

- Video camera surveillance to evaluate feed attraction and consumption
- Biometrics carried out in the nursery (before stocking) and at least once before a change in season in grow-out (one enclosure per size group)
- Weekly TRL counts to determine feed conversion rates (FCR), weekly growth, biomass per week and daily ration
- R&D hydroacustics (JCU)
- Commercial feed trials (nursery)

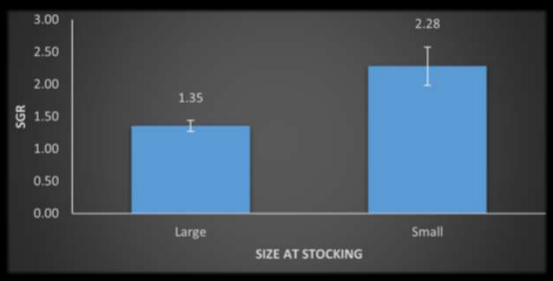
JCU Tropical Aquafeed Innovations Lab

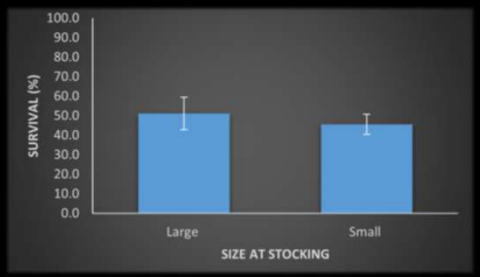
22

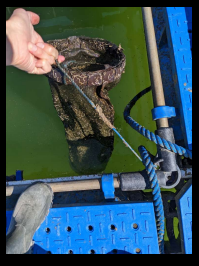
ornatas

23

WP5: Test stocking density and biofouling management







24

WP5: Test stocking density and biofouling management

Lobster Production performance

- TRL performance in summer vs winter conditions
  - 9 stocking events
- Information obtained for production model and system improvements

Summer conditions 2022_23

Summer conditions 2023_24

- Improved water quality conditions

Winter conditions 2023

- Good growth in winter
- Smaller juveniles grew faster than the larger juveniles

25

WP6: Evaluate product quality for a diversified market

THREE CAPES MARKETING BY JSJ SEAFOOD

Honey & Fox

- Global market constantly being monitored
- Provenance Technologies report
- Demand research
- Consumer research. Internal taste testing and chef's table workshop to evaluate product quality (data being analysed)

26

27

Future R&D

Across site

- Cannibalism/ behaviour
- Feeds and feeding strategies
- Health management strategies (e.g. bath treatments)

Grow-out

- Defining optimal water quality ranges – inform operations in tropical wet and dry seasons
- Offshore trials

28

The Sea Raft Project:

ornatas

Under a collaborative project funded by the CRCNA and supported by FRDC, our aim is to bring Tropical Rock Lobster sea raft grow-out to Northern Australia

Project partners: maxima the opportunity group; Three Capes Marketing by JSJ Seafood; Honey & Fox; PFG Group; University of Tasmania; IMAS Institute for Marine & Antarctic Studies

Funded by: FRDC Fisheries Research & Development Corporation; CRCNA

29

Thank you

30

Business model

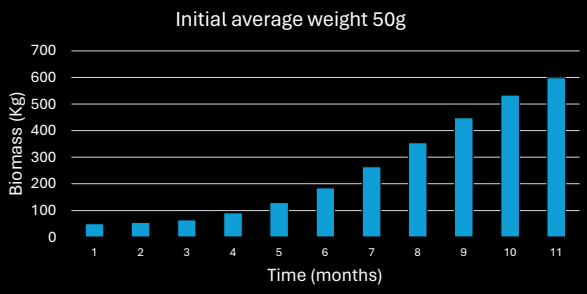
ornatas

- Initial average weight 3g

- Initial average weight 50 g

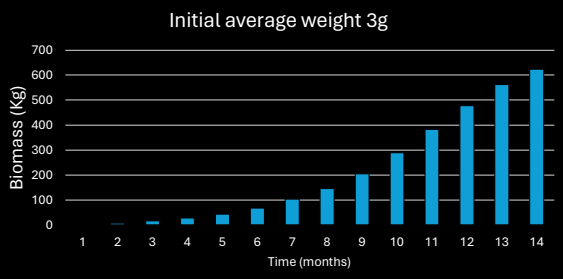
Assumptions
- 50% survival over production cycle (cannibalism)
- Growth rate (SGR) decreases with size
- No impact of seasonal change on growth

1

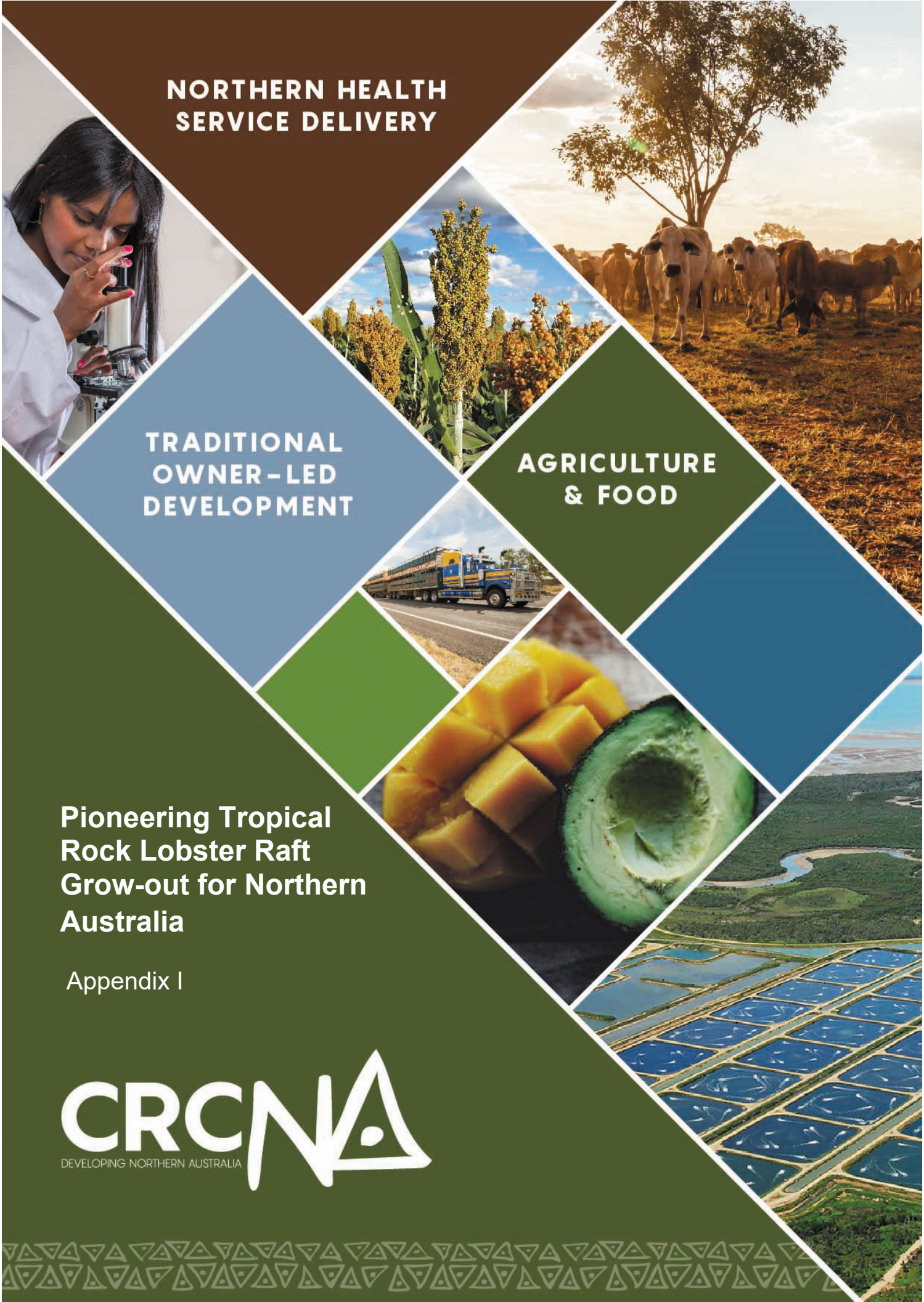


Temporal change in biomass

Time to harvest at 1.2kg
- 11 months for 50g initial size
- 14 months for 3g initial size



2



Pioneering Tropical Rock Lobster Raft Grow-out for Northern Australia

Appendix I

CRCNA
DEVELOPING NORTHERN AUSTRALIA

Work Program 6: Market ready lobster quality

Final Report May 2024

Background

Closed cycle (hatchery produced) Tropical Rock Lobster aquaculture provides the opportunity to modify culture protocols, systems, and feeds to optimise product quality for premium, high-value markets. Longer-term, beyond the scope of this study, selective breeding and biotechnologies can be applied to enhance quality and productivity further.

Work Program 6 conducted trade and consumer market acceptability trials to understand product quality expectations and specific market requirements across the supply chain. The focus was on understanding the modern consumer demand for premium rock lobsters to inform ongoing development in production and grow-out systems that would support market entry and growth, including for markets outside China.

Method

There were five activities undertaken as part of the work program.

1. Assess the impact of biofouling management and harvest time on product quality (WP6-1)
2. Undertake trade and consumer market acceptability trials, including food safety and optimisation of quality and presentation (size, shape, external colour, texture, taste, flesh colour, and resilience to handling) of sea raft produced lobsters. (WP6-2)
3. Adopt, evaluate, and, if needed, adapt provenance and branding authenticity technologies for a new Australian premium Tropical Rock Lobster aquaculture product. (WP6-3)
4. Understand the modern consumer global demand for premium rock lobsters to inform ongoing market retention and growth, including markets outside China. (WP6-4)
5. Market monitoring system established informed by demand research in this project and previous studies. (WP6-5)

Research outputs

Research activities and findings are summarised in the following reports and presentations

2022	Global Market Opportunity Review incorporating market dashboards Chinese importer in-depth interviews Chinese consumer social listening study Market monitoring report (1). Commercial in confidence
2023	Provenance Technologies Report Consumer Survey - China, Korea and Singapore
2024	Ornatas Farm Visit and Product Tasting Report(2024) incorporating in-depth interviews with chefs, retailers and supply chain participants. Market monitor report (2). Commercial in confidence

Summary of findings

Global Market Opportunity Review Incorporating Market Dashboards

Six markets have been identified as suitable for a diversified market development strategy for Ornatas to nurture as production is ramped up. These are:

- **China,** where a gap in the market for a specific sized lobster has been identified.
- **Hong Kong** is a staging post for building awareness in Southern China, where tropical lobsters are most popular.
- The **USA** has an established market for frozen lobster tails and where consumers value sustainability credentials and proof of provenance.
- **Singapore** and **Taiwan** are emerging markets, heavily influenced by Chinese culture and where Australia's rock lobsters are valued as a premium offering.
- As an additional emerging market, **Korea** is subject to market access issues being resolved to round out a market risk mitigation strategy.

Other markets such as Japan, UAE and Vietnam should be monitored; however, at this stage, the effort involved in building markets there is unlikely to achieve any significant ROI. The Australian domestic market has the potential to provide niche market opportunities and is also worth watching.

Market monitoring system

Systems and sources have been established to provide the Steering Committee with six-monthly State of the Market reports. These reports are commercial in confidence, so they have not been included in this report.

During this project, the central issue impacting markets globally has been the ongoing trade dispute between Australia and China. The nature of the global lobster market means that changes in access for one market impact the supply dynamics in other markets (in both supply and demand). While, at the time of writing this report, there are some signs the trade dispute could be resolved, we cannot assume that this will happen. More recently, another issue has arisen impacting Tropical Rock Lobster specifically. China has banned all wild-caught tropical lobsters on environmental grounds. Aquaculture products are exempt, but the definition of what meets the criteria for an aquaculture product has not been provided. Market monitoring and reporting will be ongoing after the completion of the project.

Reports: "State of the Market" Reports (commercial in confidence)

Chinese Importer (in-depth interviews)

It was difficult to get information from importers about their expectations from an aquaculture TRL product. This was due to the ongoing trade dispute between Australia and China and because there is no understanding in the market about what a "closed life cycle" product looks like and how it might perform in the market. Within this context, there is a general agreement (among China-based importers) that the primary quality characteristic of a live lobster is robustness. The key question is how long it can survive in the tank on arrival in the market. Once the product is ready for trial shipments to China, the importer interviews should be repeated and, if possible, conducted in person face to face.

Modern Chinese Consumer (desktop review)

The desktop research revealed that there are new consumer groups, new eating occasions and new sales channels that provide opportunities for market growth.

1. Consumers buy lobster species with compelling narratives. We need to craft a unique story around Ornatas Tropical Rock Lobster, highlighting the distinct qualities that set it apart from other Australian lobsters.
2. Men purchasing for their families or themselves drive most lobster discussions online and are a primary audience for marketing. However, there are new consumer groups, such as affluent singles and parents who only buy the best for their child, providing opportunities for market growth.
3. Chinese New Year and the Mid-Autumn festival account for over 50% of annual high-end seafood sales; meticulous planning and consistent marketing is required to make the most of these high seasons.
4. COVID-19 has left Chinese consumers particularly sensitive towards matters of health. While lobster is regarded as one of the healthiest foods, many consumers now are weary of food/meat that may contain diseases/viruses, etc. Reassurance to consumers is necessary**.**
5. Chefs set the high bar for brand tonality, while the more down-to-earth, personable bloggers bring the brand close to consumers. Leverage male influencers on Douyin and Bilibili and female influencers on Little Red Book.

Report: Modern Chinese Consumer (desktop research) Report (2022). Appendix J.

Provenance Technologies

The increasing physical and psychological distance between consumers and the food source drives the growth in demand for food provenance. Consumers want to know where the food they eat comes from, who is producing it and how it is produced. By communicating the provenance of produce and value-added products, including how it was produced and transported, farmers and producers may obtain a competitive edge over their rivals and, potentially, the ability to access niche markets and higher profits that might typically be unattainable.

Various technologies are available to help communicate throughout the supply chain, increasing consumer knowledge of a product's provenance and enabling producers to differentiate themself in competitive markets effectively.

Consumers expect the provenance story to be backed up with authenticity, which means the product is genuine - it is "as described". It appears that the more virtual consumers' lives get, the more something genuine is desired. Modern consumers demand products that reflect this renewed desire for what is authentic.

A range of technologies available to Ornatas was reviewed and summarised in a report, together with a decision tree to help select the most appropriate technology for the circumstances (product and market). Ornatas should revisit provenance technologies when they are closer to selling products and be guided by their customers on what is most suitable.

Report: The Provenance Technology Report (2023). Appendix L

Consumer Survey (China, Singapore, Korea)

Daxue, a specialist Asian market research agency, conducted a consumer survey in three markets: China (n=300), South Korea (n=50) and Singapore (n=50). All respondents were the decision makers purchasing lobster while dining out in the last 12 months and were the decision maker.

While the sample sizes for Singapore and Korea are too small to draw any conclusions, there were some indications of similarities and differences across all three markets that warrant further investigation when the actual product is available for testing in those markets.

The key findings are summarized below:

Comparisons across the three markets

The driver for purchase varies in each market. Chinese consumers say that they are purchasing lobster for taste, while Koreans report purchasing to create a special moment, while Singapore consumers mostly purchase to treat themselves.

Chinese consumers are more likely to choose wild-caught lobsters than Koreans, where the consumers seem to have no strong preferences. In contrast, Singaporean consumers prefer farmed lobsters – potentially due to sustainability concerns. Consumers in Shanghai also appear to prefer farmed over wild (note sample size is too small to draw a firm conclusion).

Unlike Chinese consumers, Singaporean and Korean consumers consider price, head vs tail size as top criteria over “signs of life” (vigorousness). When choosing based on colour, Chinese consumers were drawn to the more orange-coloured lobster, Koreans were drawn towards less variable colour, while Singaporeans liked the brighter blue colours.

Singapore

There is a more balanced distribution of preferences regarding lobster colouration (indicating a higher level of product knowledge or acceptance of variety), with 50% of respondents claiming they would "definitely" buy “Oceanic Farmed TRL”. This means there is a high potential for product acceptance with few philosophical barriers. Singapore's geographic proximity and logistics/supply-chain hub expertise offer advantages as a hub for other Asian markets. At the same time, its affluent consumer base and willingness to pay for high-quality seafood are also beneficial.

Market players will more likely take smaller quantities, and it is a more accessible market to work in compared to China (language, culture, regulations), yet it has many similarities to it. During and following Covid 19, Singapore has experienced a large influx of mainland Chinese, mainly from Southern China, where Tropical Lobster is typically consumed. Even before that, Singapore had influenced food trends in China, so marketing strategies could take advantage of this by testing them before launching in China.

China

The size of the China market inherently offers high potential. But, given a choice, consumers strongly prefer wild-caught lobsters. This means more effort will be needed to educate the market about how Ornatas' production methods differ from others. Ornatas could however decide to remain silent on that product attribute, at least initially, and focus on solid sustainability messaging. Concerns around the price and taste comparison to wild lobsters were raised, suggesting a need to consider pricing strategies and quality assurance in this market.

Korea

South Korean consumers showed a balanced preference between wild-caught and farmed lobsters. Regular consumers also showed a stronger likelihood of buying farmed TRL. However, Korean consumers also preferred a slightly larger lobster size.

Reports: Consumer Survey Findings Report (May 2023). Appendix K.

Product taste testing and quality assessment

An initial taste testing session was conducted with Ornatas staff and Board members in October 2023. From the total number of people surveyed (n=20), 55% had tasted TRL before. There was variation in preference for steamed or sashimi, with slight differences between experienced and non-experienced tasters. Overall, the steamed grow-out and wild-caught lobster held onsite was favoured over frozen tails. There was no off-flavour noted in any of the samples. The grow-out and wild-caught lobster received similar preference results.

Following this taste testing and the consumer survey findings, a small group of Australian-based end users (chefs and retailers) and seafood supply chain professionals were invited to visit the Ornatas facility in Toomulla in March 2024. Participants completed a survey on the criteria they use to determine the quality of lobsters. Then, they were interviewed to understand how they perceived the quality and taste of the Ornatas product they had witnessed harvested from the sea rafts.

Like the first Ornatas taste test session in October 2023, these experts agreed that there was no discernible difference in taste between the Ornatas product and the wild product they were used to. All were impressed with the robustness/liveliness of the animals. The product was prepared as sashimi, in Chinese style, lightly steamed and in a miso soup. The product performed well in all circumstances, and no off-flavours were detected.

Reports: Ornatas Taste Test Report (October 2023), Ornatas farm visit and product tasting report (March 2024)

Recommendations

Harvesting capabilities and Supply Chain Partnerships

- Focus on building capabilities in harvesting, packing, and transporting live and dead products to market. This can be done by linking to the facilities and expertise of Torres Straits Seafood and others.
- Initially, work on collaborating with existing partners to monitor and document trial shipments to Cairns and Townsville, ensuring the survival of animals during transportation.
- Survivability of the animal is paramount, and robustness on arrival is one of the key considerations of buyers so creating SOPs for product handling from farm to plate and train teams on the farm.

Collaborate with chefs (as influencers) to create user guidelines

- To ensure the end user's needs, which are of utmost importance, are met, we recommend creating a best practice guide to handling and cooking methods, taking a 100% product utilisation approach.
- This work should be done with a small chef panel (work with Umar Nguyen and start with the chefs who attended the farm tour).

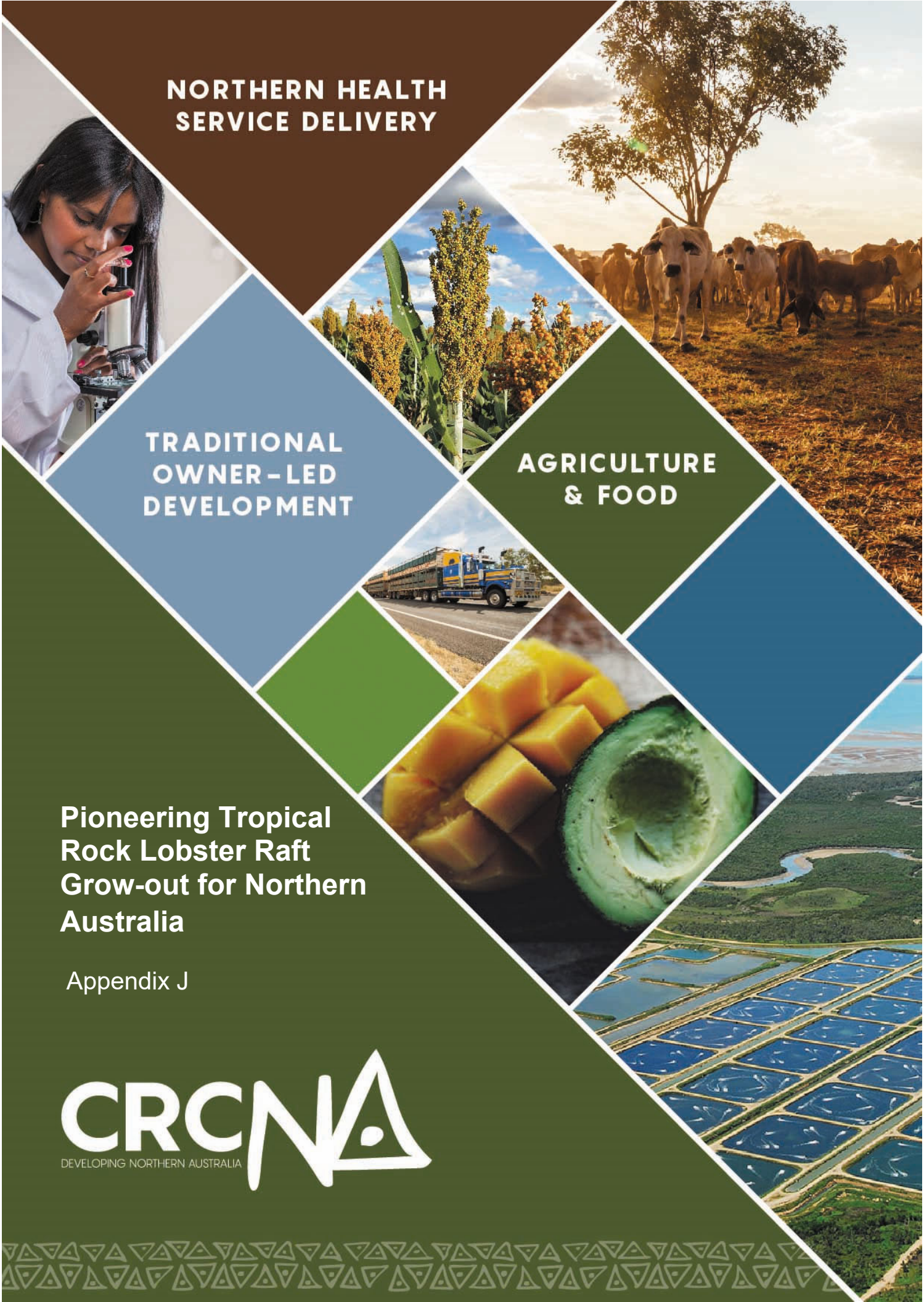
- The second stage would be to trial and evaluate dishes on specific restaurant menus and include buyer, chef, and customer responses to those.

Market Education Collateral

- As a “world first”, you can set the product and positioning parameters to make it distinctive in the market.
- Using the preparatory market research undertaken in the CRCNA/FRDC funded project, create an engaging product provenance story (place, process, planet, proof) emphasising sustainability, premium quality, Australian origin, and delicious taste.
- Create market education materials, including handling and quality management and preparation guides.
- Develop and implement a training program for the key market players so that they can tell your story the way you want it to be told.
- Evaluate market education efforts and experiment with authenticity and provenance technologies (e.g., QR codes, track and trace) to underpin these.

Market Entry Strategy

- While production volume is low and unpredictable, we suggest that the product only be marketed in the local region (Townsville and Cairns). The small volume, handled correctly and coupled with premium branding and market positioning, will likely achieve price premiums.
- This allows you to grow (time, people, and finances), establish processes, and train team members, supply chain, and market partners.
- This local approach would be followed by a phased expansion, starting with premium markets in Brisbane, Sydney, and Melbourne.
- Once production and distribution capabilities are firmly established, consider venturing into selected international markets, with China at the top of the list as part of a diversified market portfolio that includes Singapore and Korea.



Pioneering Tropical Rock Lobster Raft Grow-out for Northern Australia

Appendix J

CRCNA
DEVELOPING NORTHERN AUSTRALIA

Modern Chinese consumer demand for premium rock lobsters

Desktop research findings

(Presentation to SC July 2022)

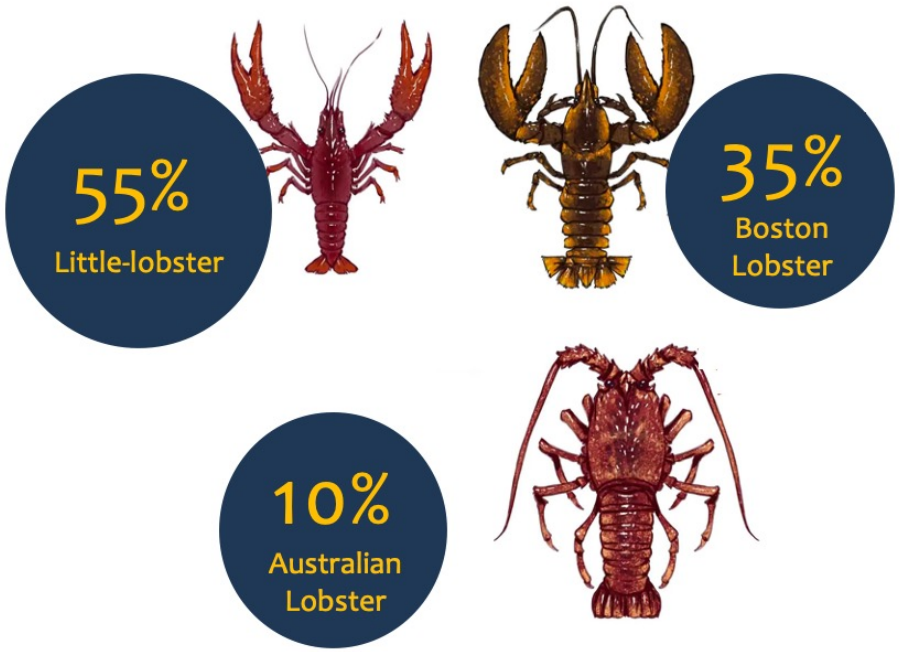
Honey & Fox

Seafood market merchant: “The most expensive seafood here is Ao Long (Australian Lobster).”

Lobster perception in China

Sources: SUP China, e-commerce China, TMALL search index, Zhihu

- Red Swamp Crayfish (aka Little Lobster) have stormed into China's culinary scene and have found their way into the hearts of Chinese foodies. From being a popular choice for spicy dishes paired with beer on summer nights to being specially processed into seasoned and cooked half lobster tails, they have proven their versatility in the market.
- The American spiny lobster, aka Boston Lobster, is the best-known and most popular imported lobster. It is readily available through e-commerce platforms with relatively affordable price points.
- Australian Rock Lobster is still considered a luxury food for Chinese formal banquets and celebrations. With the current trade dispute, New Zealand lobster has filled the market vacuum.



BILIBILI

Most played video featuring Australian lobster

Title: What's the difference between a ¥2500 (A$ 543.5) Australian lobster and a ¥500 (A$108.7) Colored lobster?

Play: 3.42M
Engage: 16k

Witty comment: In the presence of Ao Long, the Colored lobster is like a petty little brother.

The blogger (4M-followers) recorded himself steaming and eating the two lobsters. The brevity of cooking technique amplified the delicious looks of the lobsters, much envied by netizens.

However, as much as magnificent the lobsters looked, knowledgeable netizens commented that the Ao Long that the author bought was not really the precious Southern Australian Rock lobster, it's American or Mexican Red Lobster. Other helped clarify the differences between different kinds of lobsters in the comments.

Similar situations happen in other lobster videos, indicating a common lack of knowledge of how to distinguish different lobsters, even to the lobster lovers.

Source: Roo Life Group

海底捞鲜批发店 HAI DI LAO XIAN (海底捞鲜)

Positioning & Story

Since 2017, Hai Di Lao Xian has been selling a variety of JiangSu Special seafoods and imported seafood via their own Taobao operations. They sell iced-fresh Boston Lobster and King crab. Although Australian Lobster is not on the selling list, they use'Australian Lobster' as key words to attract customers who take interest in Australian lobsters.

Key Messaging

Iced-fresh preserved process to lock nutrition & freshness.

Caught Deep Sea, Frozen.

Naturally Grown in the clean deep sea for a succulent texture.

Superior Cold-chain delivery.

Top Seller

Iced-fresh Boston Lobster 2000g

¥ 430 (A$ 93.5)

https://item.taobao.com/item.htm?spm=a1z10.1-c.w4023-23515061304.4.5961acbamApeup&id=639879679269

盒马 HE MA FRESH (盒马生鲜)

Positioning & Story

Hema Fresh is a fresh product retail chain completely reconstructed by Alibaba for offline supermarkets from 2015. It is supermarket, a restaurant and fresh market 3 in 1. Consumers can purchase at the store or place orders on the Hema App. And one of Hema's biggest features is fast delivery: within 3 kilometers of its stores, goods can be delivered to your door in 30 minutes.

Key Messaging

Fine living supported by HEMA tech with more personalised experience.

Fresh every moment

Get whatever you want anywhere

One-stop shopping offline and online app

Turning Cooking into entertainment

Top Seller

Ready-to-eat Little Lobster 750g*2

¥ 128 (A$ 27.8)

Malaysia iced-fresh Green Lobster 500g-750g

¥ 199 (A$ 43.3)

Source: Roo Life Group

KING OF YUXIAN (御鲜之王)

Positioning & Story

Founded in 2005, Yuxian focuses on the development and production of high-end seafood products such as American Lobster and abalone and king crab. In 2019, the brand launched its fresh American Lobster on JD platform – and they claimed that Lobster will be still alive delivered to doorstep supported by superior supply chain and 25 warehouses.

Key Messaging

Convenient, High-End, Affordable,
Easy to cook, big American Lobster.

Naturally Grown in the clean deep sea in Canada.

Nutrition value esteem value

Superior logistic services by 25 warehouses

Top Seller

Live Boston Lobster 1000-1200g

¥ 399 (A$ 86.8)

https://item.jd.com/63104659046.html

LE SHI GANG (乐食港)

Positioning & Story

Leshigang launched their online retail business in 2015 on JD platform. They sell a combination of plain, unprocessed live and iced seafood. Le Shi Gang takes great care in carefully explaining the intricate details of preciousness and naturalness of live Boston lobster mainly.
Superior logistics and order fulfillment are some of their key value propositions. The company takes pride in utilizing 25 warehouses across China and that consumers are able to receive seafood live in no time.

Key Messaging

Naturally grown in the deep sea. Caught in clean seawater to ensure freshness and quality.

Technology of perserving to maintain freshness, nutrition and taste.

Superior logistics services by 25 warehouse

Cooking Tutorial from Chinese chef

Top Seller

Live Boston Lobster 750g

¥ 299 (A$ 65)

https://item.jd.com/100013902577.html#crumb-wrap

Source: Roo Life Group

Consumer profiles

Source: Roo Life Group

Affluent Family pre-marriage Preparation
For wedding

- Parents of affluent family or young couples prepare for wedding ceremony.
- Middle class in first/second–tier cities.

Bachelors/ single young ladies
For themselves

- 25–35 years old, first – tier cities
- Single or live by themselves.
- Lead a healthy and premium lifestyle.

B2B buyers
For customers

- Chefs/procurement managers at upscale restaurants and hotels
- For engagement parties, wedding ceremonies, family celebrations, business dinners, company annual parties etc.

Buying situations

Source: Roo Life Group

Wedding/ Banquet

Tasty cuisine for your children and loved ones

Special treat to family/friends' reunion

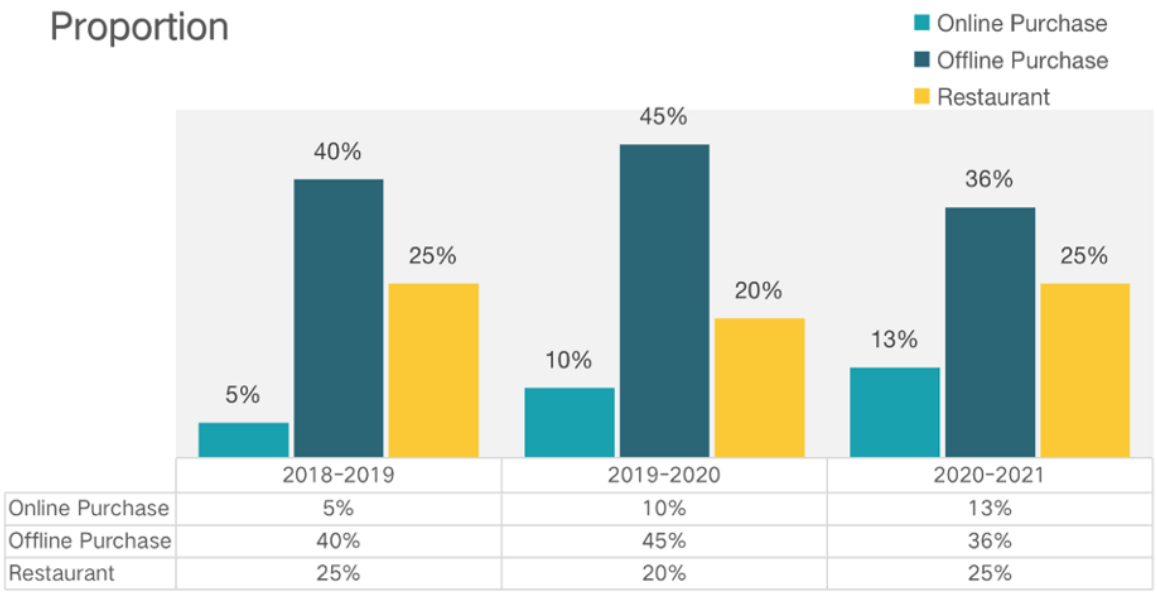
Chinese celebration gifting for significant connections

Just want to indulge myself a little bit

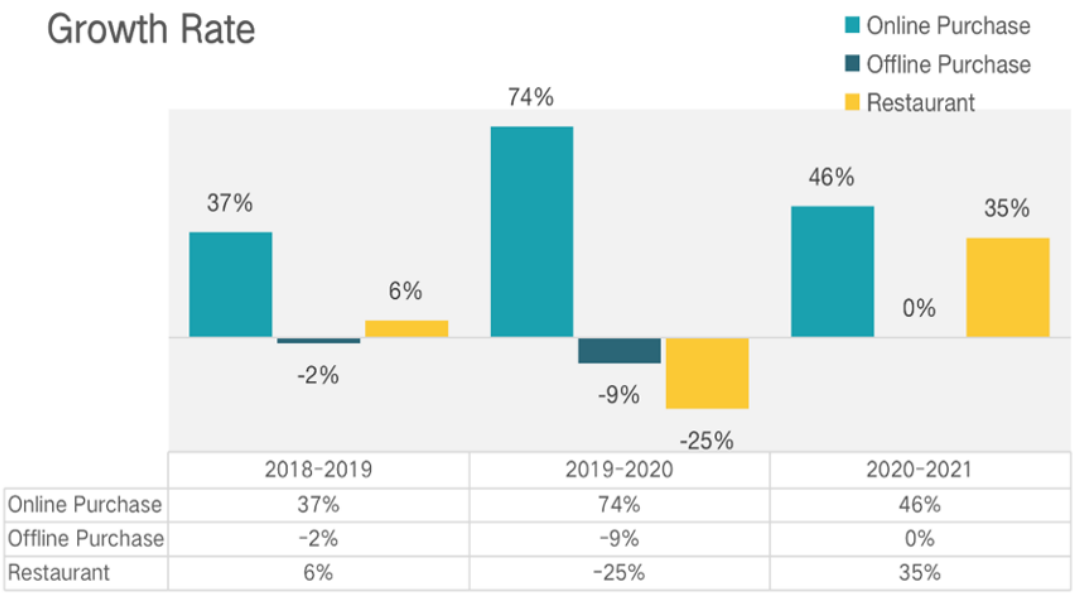
Purchase Channels

Source: Roo Life Group

CHANGE IN PURCHASING CHANNELS



	2018-2019	2019-2020	2020-2021
Online Purchase	5%	10%	13%
Offline Purchase	40%	45%	36%
Restaurant	25%	20%	25%

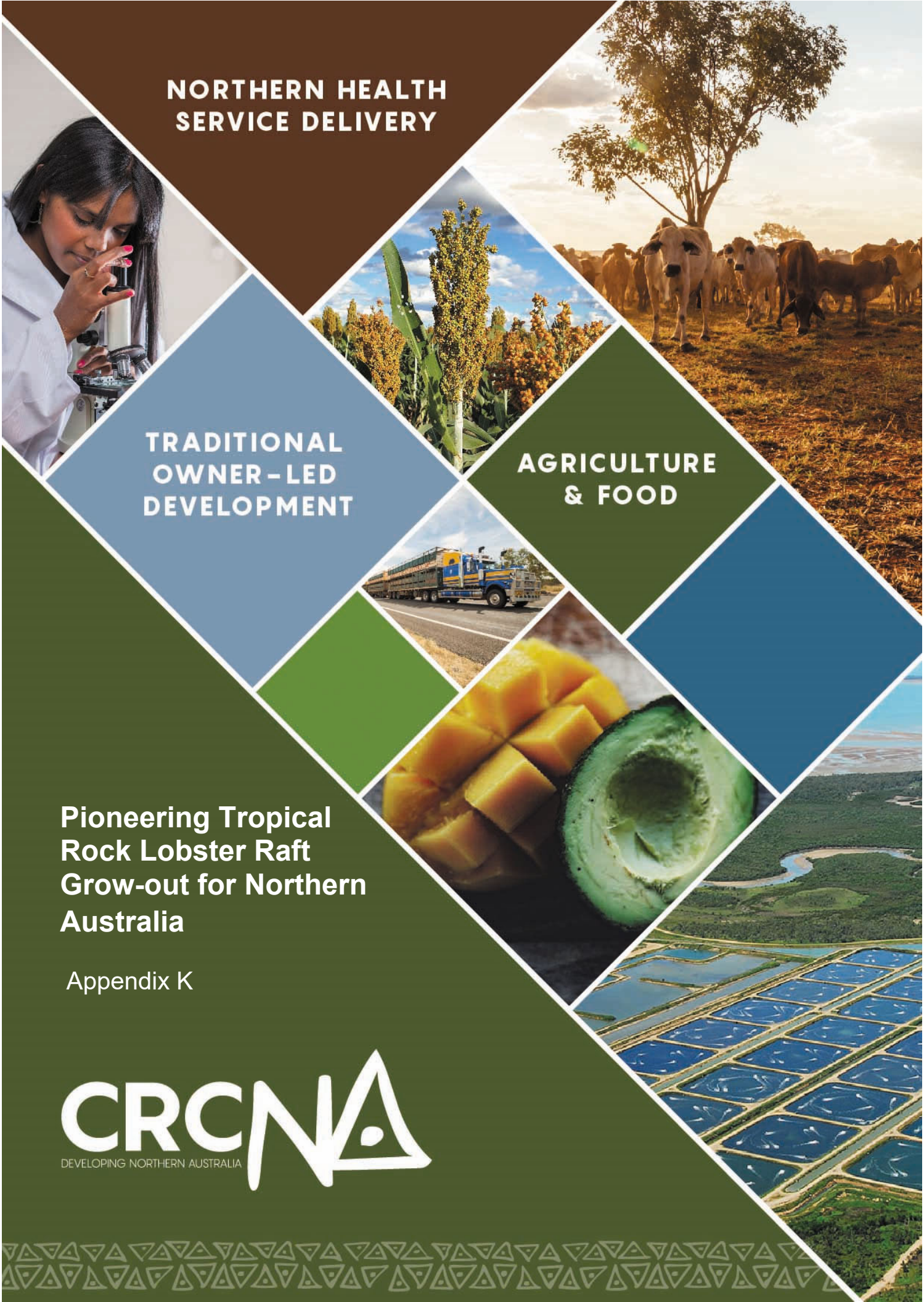


	2018-2019	2019-2020	2020-2021
Online Purchase	37%	74%	46%
Offline Purchase	-2%	-9%	0%
Restaurant	6%	-25%	35%

Online Purchase:
Convenient and safe during Covid-19 period. Group purchase in community/e-com ecosystem

Fresh market/large one stop supermarket chain, HEMA, Sam's, Wal-Mart.

Dining in fine restaurants： high-end hotel cafeterias, Chinese and Japanese restaurants.



Pioneering Tropical Rock Lobster Raft Grow-out for Northern Australia

Appendix K

CRCNA
DEVELOPING NORTHERN AUSTRALIA

Honey & Fox

Ornatas Consumer Research Report

May/June 2023

Daxue, a specialist Asian market research agency, conducted a consumer survey in three markets, China (n=300), South Korea (n=50) and Singapore (n=50). All respondents had purchased lobster while dining out in the last 12 months and were the decision maker.

This report summarises the key findings and identifies the implications of these for developing market entry strategies. A full copy of the consumer survey report is attached (Attachment 1).

Market comparisons

- The driver for purchase varies in each country. Chinese consumers purchase lobster for taste, while most Korean purchase to create a special moment, and Singapore consumers mostly purchase to treat themselves.
- Chinese consumers are more likely to choose wild-caught lobsters than South Korean, where the consumers seem to have no strong preferences, while Singaporean consumers prefer farmed lobsters – potentially due to sustainability concerns.
- Consumers in Shanghai also appear to prefer farmed product over wild (note sample size is too small to draw a firm conclusion).
- Unlike Chinese consumers, Singaporean and Korean consumers consider price, head vs tail size as top criteria over “signs of life” (vigorousness)
- When choosing based on colour, Chinese consumers were drawn to the more orange-coloured lobster (Pic 6), Koreans were drawn towards less variable colour (Pic 1), while Singaporeans liked the brighter blue colours (Pics 2, 5 and 7)

Singapore

There is a more balanced distribution of preferences regarding lobster colouration (indicating a higher level of product knowledge or acceptance of variety), with 50% of respondents claiming they would "definitely" buy “Oceanic Farmed TRL”. This means there is a high potential for product acceptance with few philosophical barriers.

Singapore could act as a potential jump-off point for future Asian market development, at least in the short term. Singapore's geographic proximity and logistics/supply-chain hub expertise offer advantages, while its affluent consumer base and willingness to pay for high-quality seafood are also beneficial.

Market players will more likely take smaller quantities, and it is an easier market to work in compared to China (language, culture, regulations), yet it has many similarities to it. During and following Covid 19, Singapore has experienced a large influx of mainland Chinese

© Honey & Fox 2023

particularly from Southern China, where Tropical Lobster is typically consumed. Even before that, Singapore has influenced food trends in China, so marketing strategies could take advantage of this, testing them before launching in China.

While Singapore could be an opportunistic market for Ornatas, our discussions with Singapore importers suggest that Singapore is highly price sensitive. The fine dining sector appears not to be quite as price sensitive, but most of the volume sales by importers are driven by the price and availability of lobster. For example, last week (July 3, 2023), Tropical lobster, Homarus and Ornatus mixed, mainly under 500gm, was sold at $USD25 per kg. Lots of cheaper farmed product is pouring into Asian markets now, all under 1kg, providing a competitive advantage for Wild Australian TRL enabling much higher prices to be achieved.

In summary, we suggest that Singapore could form part of a diversified market mix once sales and volumes are established in China.

China

The size of the China market inherently offers high potential. But, given a choice, consumers demonstrate a strong preference for wild-caught lobsters. This means significant effort will be needed to educate the market about how Ornatas' production methods differ from others. Ornatas could decide, at least initially, to remain silent on that product attribute, and focus on other provenance story elements, including strong sustainability messaging.

Concerns around the price and taste comparison to wild lobsters were raised, suggesting a need to consider pricing strategies and quality assurance in this market.

South Korea:

South Korean consumers showed a balanced preference between wild-caught and farmed lobsters. Regular consumers also showed a stronger likelihood of buying farmed TRL. However, Korean consumers also preferred a slightly larger lobster size.

Further research

The consumer preference data is interesting with three issues standing out as being worthy of further investigation:

1. **Marketing messaging**: wild vs farmed in the more mature markets. The data around Shanghai and farmed needs further investigation with a larger sample size and perhaps a series of questions to understand where farmed lobster would sit compared to wild. From a marketing messaging point of view, it would seem sensible to focus the Ornatas provenance story on other elements and remain silent on the production method until we understand more.
2. **Colour preference:** the colour preference information is not what we expected and again needs further investigation to understand whether this finding stands up with larger sample sizes and better photos.
3. **China regional preferences**: go deeper into regional differences in China to understand the implications for market entry and development strategies

© Honey & Fox 2023

Recommendations and Next Steps

It is recommended that:

1. Further market research be conducted to investigate attitudes to farmed lobster and implications for marketing messages.
2. A high-quality lobster photo library be established that shows a range of colours for TRL. This can then be used to further investigate colour preferences within China and Singapore.
3. Develop, a good photo and video library, showing behind-the-scenes visuals of Ornatas's progress. This will be useful for further market research and help keep the market interested until the product is available.
4. Update existing and develop new market education materials tailored for specific markets. These materials should focus on building awareness of the Ornatas provenance story (people, product, place and process) as a precursor to a market entry/launch.
5. Investigate and trial provenance technologies for incorporation into branding and market education efforts.
6. A market visit to China and Singapore be undertaken in 2024 to further investigate the role that the market could play in Ornatas' diversified market strategy. This would need to involve:
    a. Conducting in-depth interviews with potential partners in the food service sector that could reveal important insights for product development and market education materials. The interviews would deepen our understanding of:
        i. beliefs about the environmental impact, ethical considerations, and the potential effects of messaging about farmed lobster vs wild harvest;
        ii. price sensitivity and what is valued in a premium tropical lobster offering
        iii. the taste profile of Ornatas lobsters, compared to others on the market (including wild) and cooking styles to suit
        iv. the most effective channels for promoting lobsters
    b. Exploring partnership opportunities with high-end food service channels that could provide a launchpad for the product and position it as a premium offering.
    c. Identifying potential partners and distributors in Singapore, assessing the necessary logistics, cold chain, and tanking requirements (including in restaurants), and understanding any potential challenges in exporting live TRL from Australia to Singapore.
    d. understanding import regulations and food safety requirements specific to Singapore to ensure that the production methods do not add different requirements to those for wild-caught products.
7. Undertake further market research in China to understand the regional/city differences. This should be done via an in-market visit once the Ornatas product is available to taste.

© Honey & Fox 2023

daxueconsulting
beijing shanghai

Honey & Fox

ornatas

Daxue consulting for Ornatas

Preference survey for lobster consumers in China, Singapore and Korea

Daxue team: Thibaud Andre, Lisa Zhang
May 2023

© Copyright 2023 – Daxue Consulting

Survey results - Methodology

We surveyed 400 respondents in Mainland China, Korea and Singapore

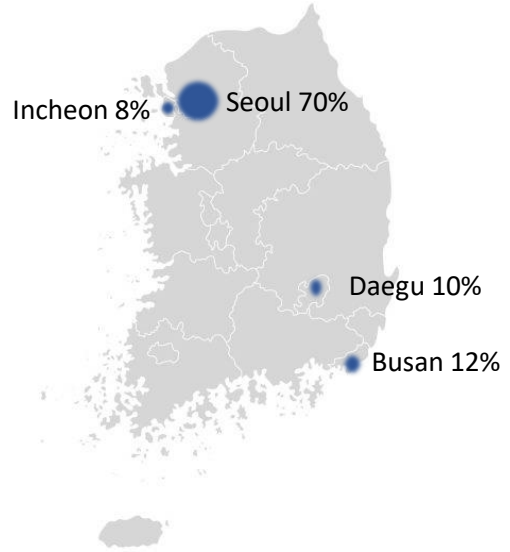
300 respondents from Mainland China

Not expatriates, only local consumers

Sample limited to tier-1 cities in the South and East regions of Mainland China.

50 respondents from South Korea

Not expatriates, only local consumers



50 respondents from Singapore

Only local consumers or expatriates living in Singapore for at least 4 years

© Copyright 2023 – Daxue Consulting

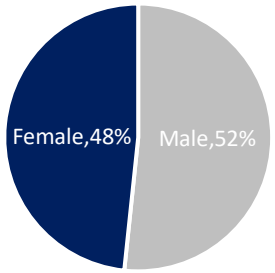
Survey results - Methodology

No specific quotas were applied for gender or age range but the organic sampling leads to a mostly equal gender distribution for decision-makers on lobster purchase

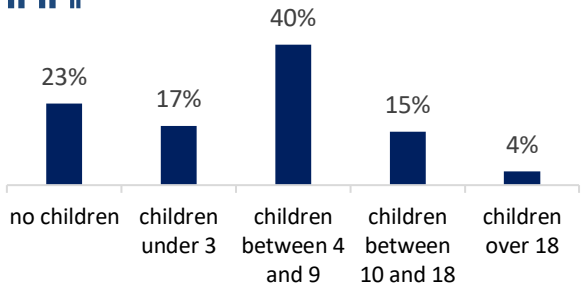
300 respondents from Mainland China

Not expatriates, only local consumers

By Gender

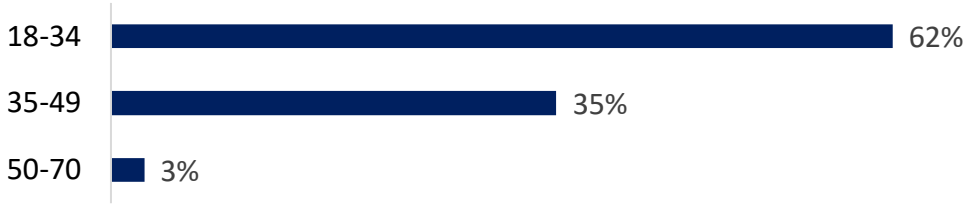


By Family Structure



No specific quotas were set for genders.

By Age



No specific quotas were set for age range – respondents under 18 year-old were screened-out.

50 respondents from South Korea

Not expatriates, only local consumers

- **50%** are men
- **32%** are single or in a couple with no child
- **44%** are between 35 and 49 YO

50 respondents from Singapore

Only local consumers or expatriates living in Singapore for at least 4 years

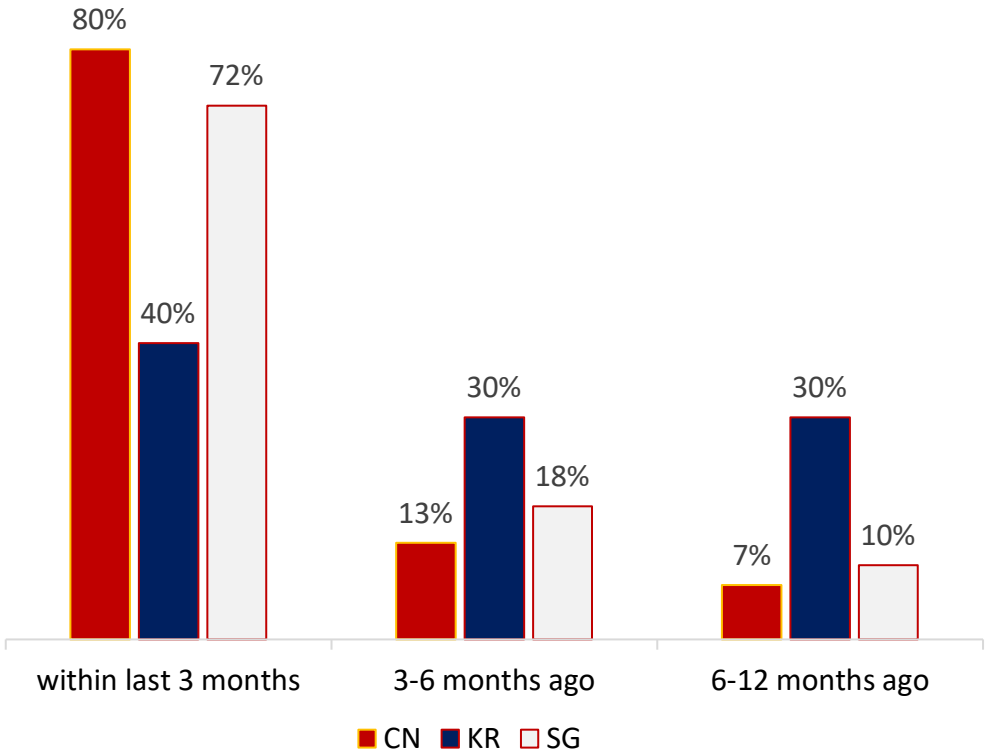
- **72%** are men
- **52%** are in a couple with a children between 4 and 9 YO
- **48%** are between 18 and 34 YO

© Copyright 2023 – Daxue Consulting

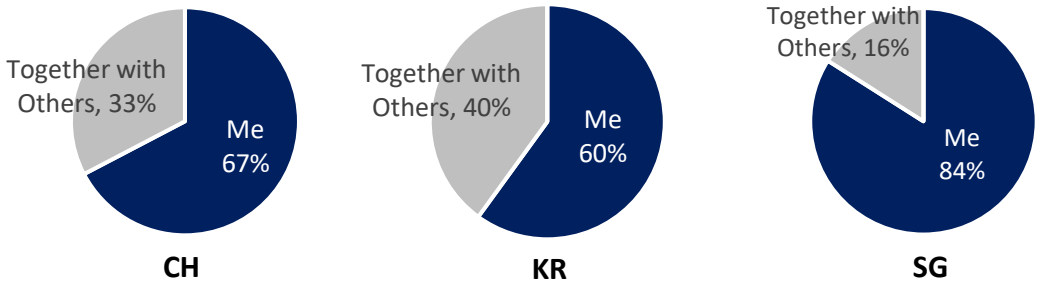
Survey results - Profiling

All the participants did purchase lobster when dining-out at least once in the last 12 months and were the decision-maker to do so. They all plan to repurchase it at some point.

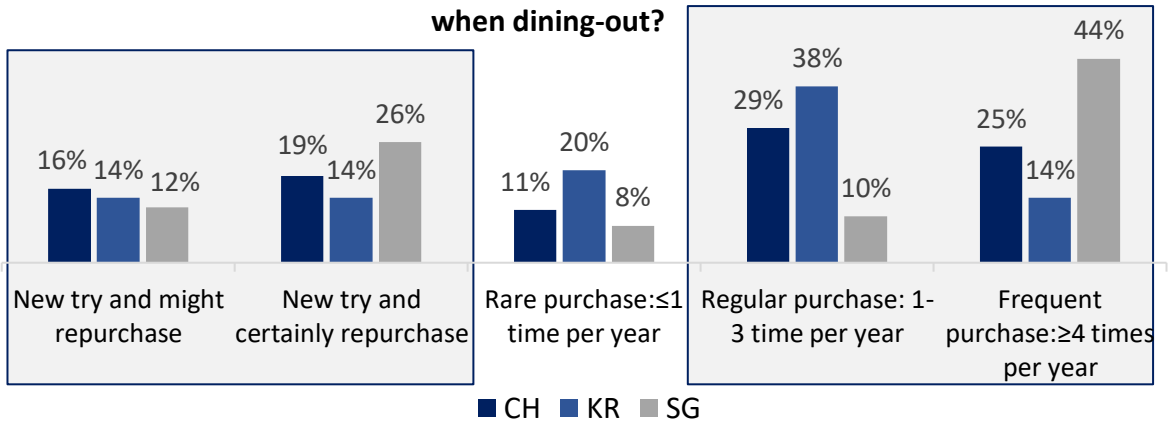
Q1.7. When did you last purchase lobster when dining out? This can be at a restaurant, café or anywhere you can purchase lobsters to eat.



Q1.8. Who made the decision to purchase the lobster when dining out?



Q1.10. How would you describe your last consumption of lobster when dining-out?



34% of the participants can be considered as new lobster consumers

66% of the participants can be considered as regular consumers

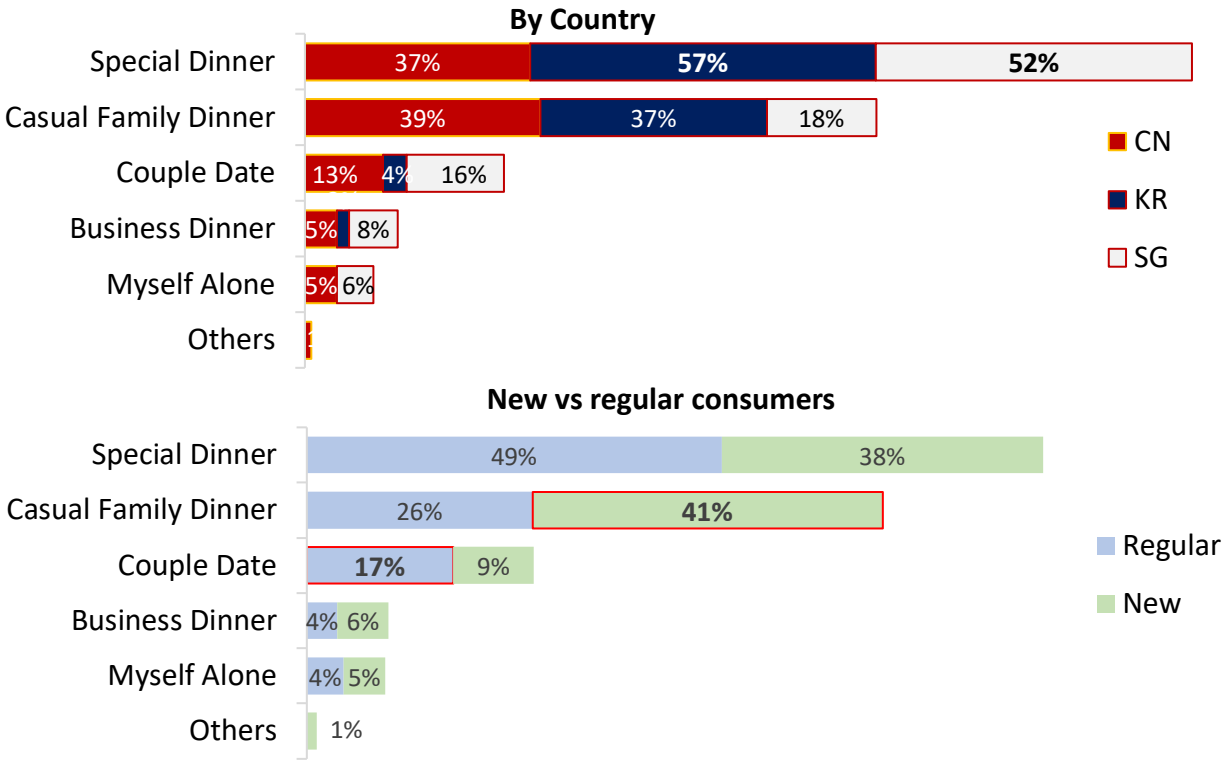
Going forward, we will also use this segmentation to cross-tab analysis some of the questions between new and regular consumers

© Copyright 2023 – Daxue Consulting

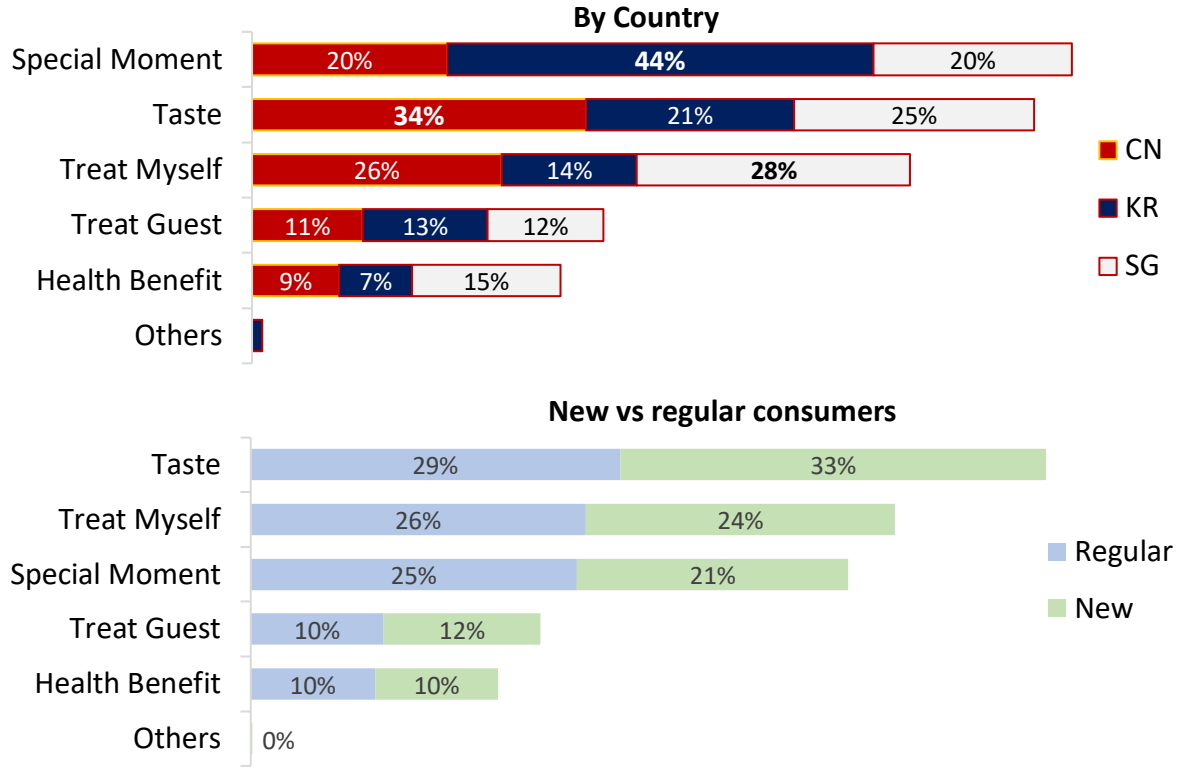
Survey results - Profiling

The main occasions to purchase are special dinners to commemorate special moments

Q2.1. What was the context of your last lobster purchase when dining out?



Q2.2. What are the main reasons you usually purchase lobster when dining out?



Special dinner (birthdays, celebration, etc) is the most common occasion to purchase lobsters, especially for Singapore and Korean consumers, in a purpose of generating special memorable moments.

When comparing new and regular consumers, it is interesting to note that **casual family dinners are actually the main driver of product discovery and not specifically special occasions**. Regular consumers are also more confident to include lobster in a couple date menu.

The driver for purchase varies in different countries. **Most Chinese consumers purchase the lobster for taste, while most Korean consumers purchase to create a special moments and Singaporean consumers mostly purchase it to treat themselves.**

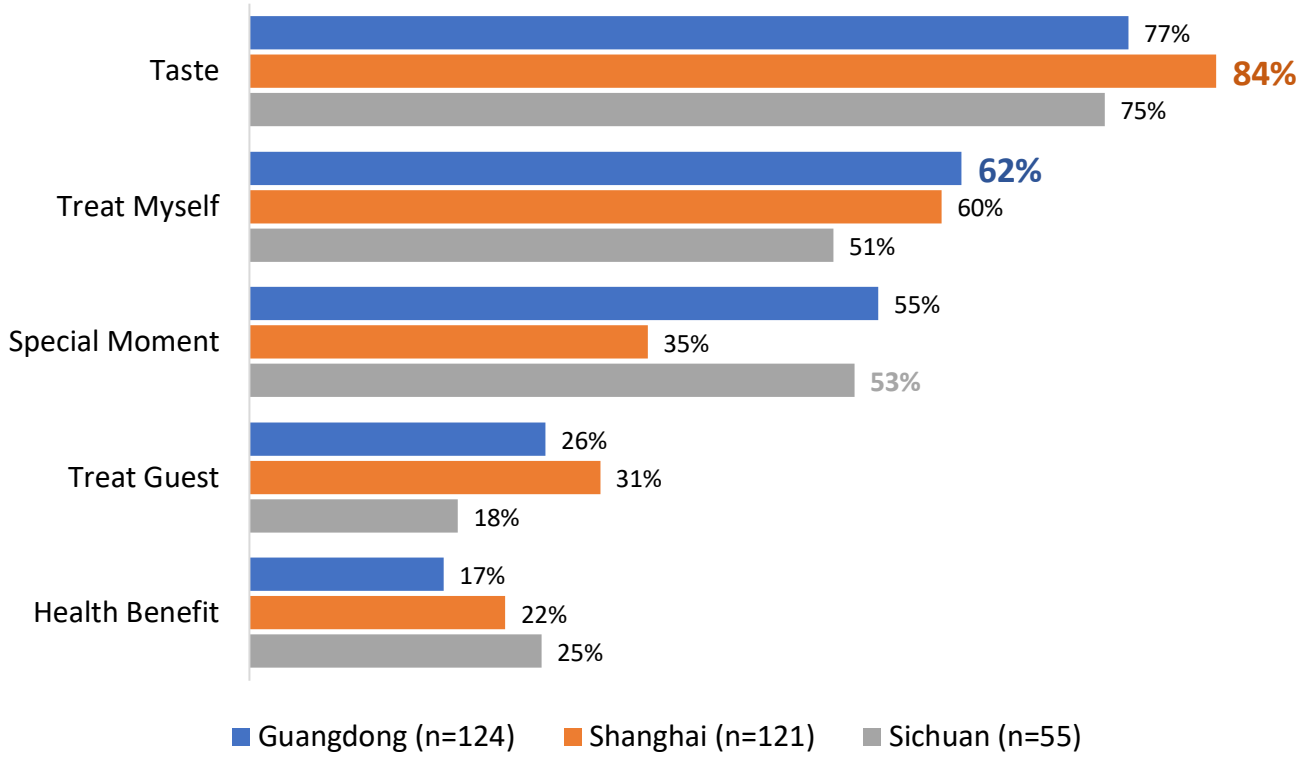
Nearly half Korean consumers regard lobsters as a special dish for memorable events.

© Copyright 2023 – Daxue Consulting

Survey results – Drivers and farming methods

[CHINA FOCUS] Slight differences in purchase reasons between cities are exhibiting a difference of perception toward lobster consumption

Q2.2. What are the main reasons you usually purchase lobster when dining out?



While taste is the top 1 driver for Chinese consumers from any of the different regions, consumers from Shanghai are especially driven by the taste of lobsters, over reasons that are less represented than in other cities like celebrating a special moment.

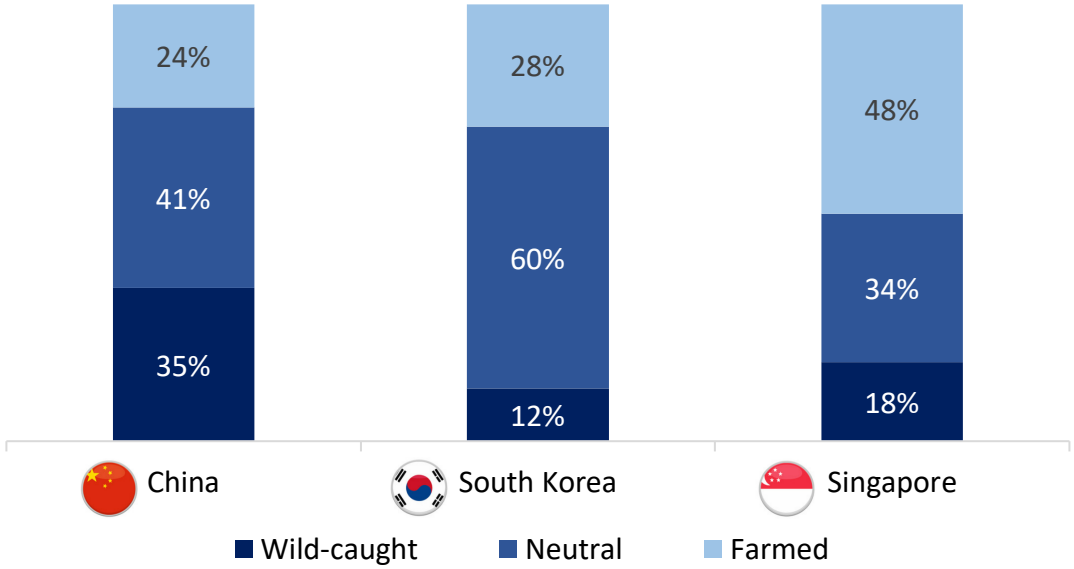
It relates to some cultural perspectives between Shanghai and provinces in the South of China, where lobsters is usually considered as a very special meal fitting celebrations or business occasions, while in Shanghai it is more appreciated for the quality of product/taste itself.

© Copyright 2023 – Daxue Consulting

Survey results – Farming methods

There is no strong consensus about the farming method. Those who prefer wild-caught lobsters are mostly influenced by a perceived better taste and richer nutrition

Q2.3. When purchasing lobster, do you prefer your lobster wild-caught or farmed?



Chinese consumers are more likely to choose wild-caught lobsters than South Korean, where the consumers seems to have no strong preferences, and Singaporean who seem to prefer farmed lobster.

It is also interesting to note that more the respondents are regular consumers, more they have defined opinion about the farming ways (less neutral respondents) while still balanced between wild-caught or farmed.

Q2.3.1. Why do you prefer wild caught over farmed?

营养更丰富 Richer nutrition
味道更鲜 More savory taste
味道更鲜 More savory taste
肉质香嫩 tender and tasty meat
天然无污染
Natural and no pollution
养殖过程有药物和激素
肉质口感更好
Drugs and hormones are used during farming
The meat tastes better
味道更鲜 More savory taste
营养更丰富 Richer nutrition
价格贵有面子
Higher price to gain face
营养更丰富
Richer nutrition
卖相好
Attractive look

> 我觉得野生的好吃,更健康。
>
> I think the wide-caught lobsters are more delicious and healthier.

> 더욱 신선하고 맛이 있기 때문입니다.
>
> Wide-caught lobsters have savory taste.

> Wild caught lobsters feed on natural feeds in the sea, hence their flesh is just natural with no chemicals are impurities that can be obtained from processed feeds. Wild caught are also more delicious.

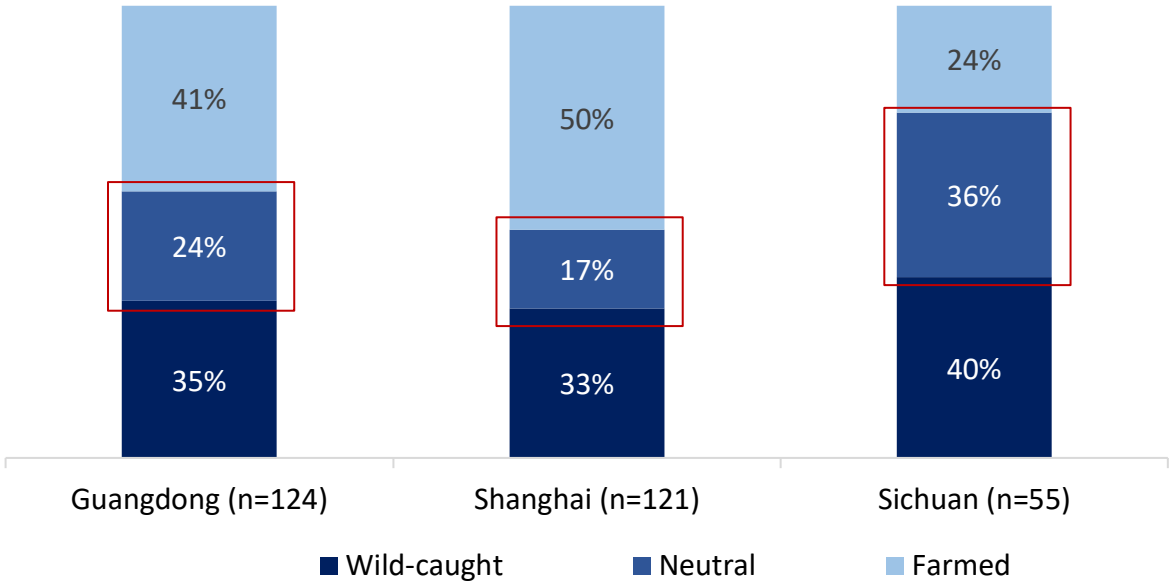
CH | KR | SG

© Copyright 2023 – Daxue Consulting

Survey results – Drivers and farming methods

[CHINA FOCUS] Shanghai respondents are favoring more farmed lobsters while Sichuan respondents are preferring wild-caught

Q2.3. When purchasing lobster, do you prefer your lobster wild-caught or farmed?



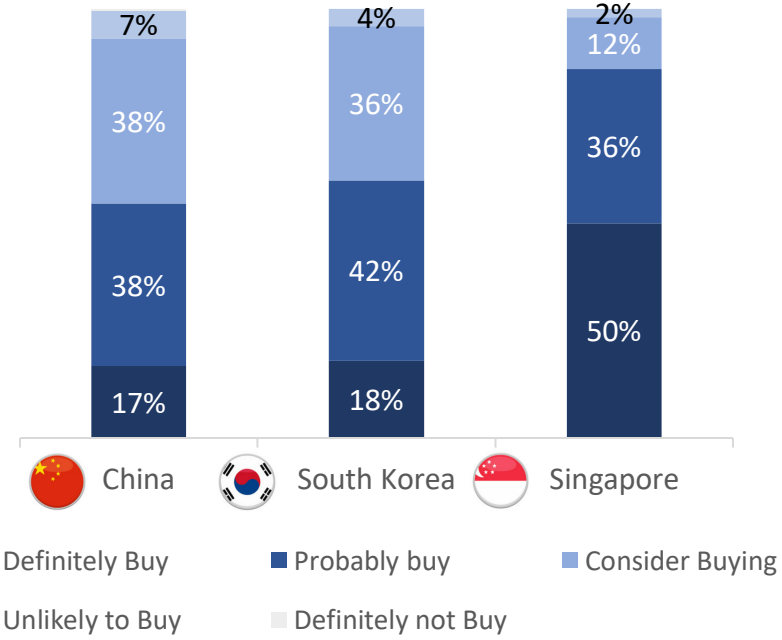
Consumers from Shanghai have stronger opinion on the farming methods. It's worth noting that they especially have higher preference on farmed lobsters, seemingly aligning with the differences identifed in purchase reasons, requiring less of a « prestige » wild-caught alternative.

© Copyright 2023 – Daxue Consulting

Survey results – Oceanic tropical rock lobster

Oceanic Tropical Rock Lobster: consumers show high willingness to purchase especially in Singapore

Q2.4. This image is an oceanic Tropical Rock Lobster. It is farmed in Australian coastal waters. How likely would you be to buy this lobster?



More than half of consumers are claiming to be a probably buyer of the tropical rock lobster. It is especially true among Singaporean respondents among which 50% claim to be « definitly buying ».

Regular consumers are more likely to buy this lobster, and such tendency is strong especially in South Korea.

Q2.5. Why are you not interested in buying this tropical rock lobster?

尺寸有点小 Small Size

没有食欲 Lack of Appetite

外观不好看 Unattractive look

价格昂贵 Expensive

长相太奇怪 Strange Look

长途运输不新鲜

Not fresh meat after long-distance transport

身体太小 Body too Small

> 价格高，购买也不太方便。
>
> I think the price is high and it’s inconvenient to purchase.

> 생김새가 특이하다.
>
> It looks so strange.

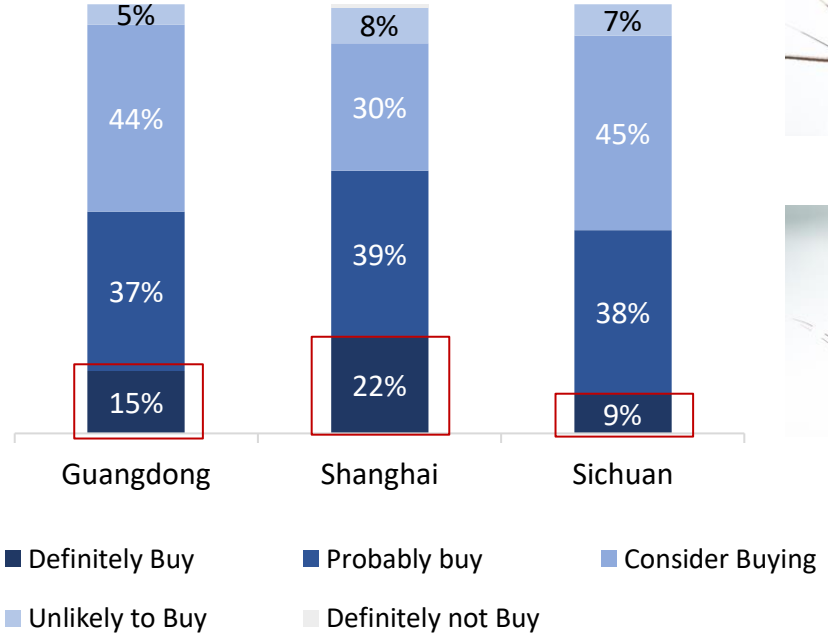
> The body is too small.

© Copyright 2023 – Daxue Consulting

Survey results – Oceanic tropical rock lobster

[CHINA FOCUS] Shanghai respondents are especially willing to purchase the Oceanic tropical rock lobster, while Sichuan respondents are showing less enthusiasm

Q2.4. This image is an oceanic Tropical Rock Lobster. It is farmed in Australian coastal waters. How likely would you be to buy this lobster?



Consumers in Shanghai show higher willingness to purchase the oceanic tropical rock lobster. 22% of them claim to buy it definitely, while consumers in Guangzhou and especially Sichuan show less strong interest of purchase.

When following the narratives of consumers in Guangdong and Sichuan purchasing more often for special occasions and business dinners, it could be extrapolated that the picture is reinsuring them less on the perception it will give for such consumption context.

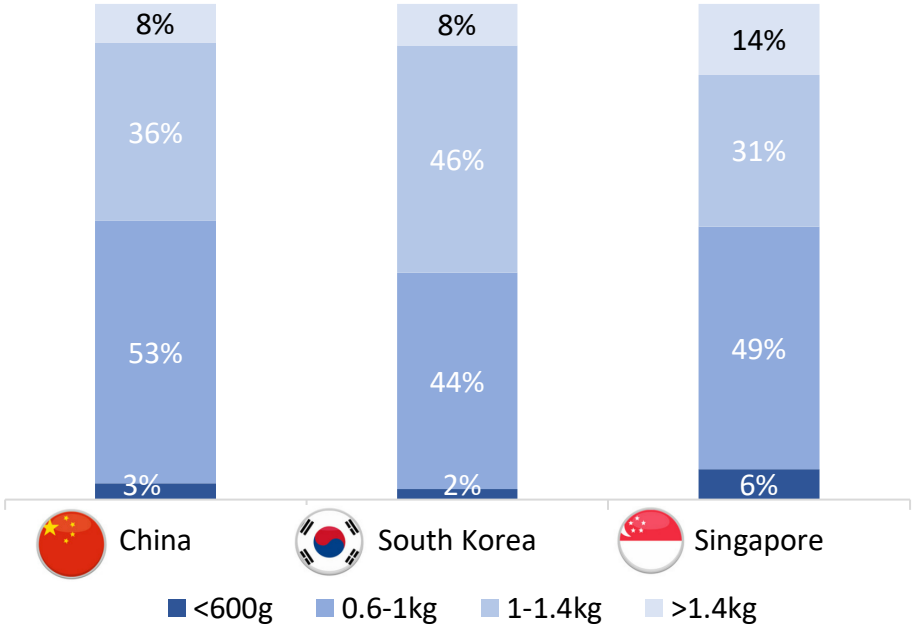
However, it is to be nuanced as the difference are not statistically significant between Shanghai and Guangdong for instance, due to the lower sample size.

© Copyright 2023 – Daxue Consulting

Survey results – Decision-making criteria

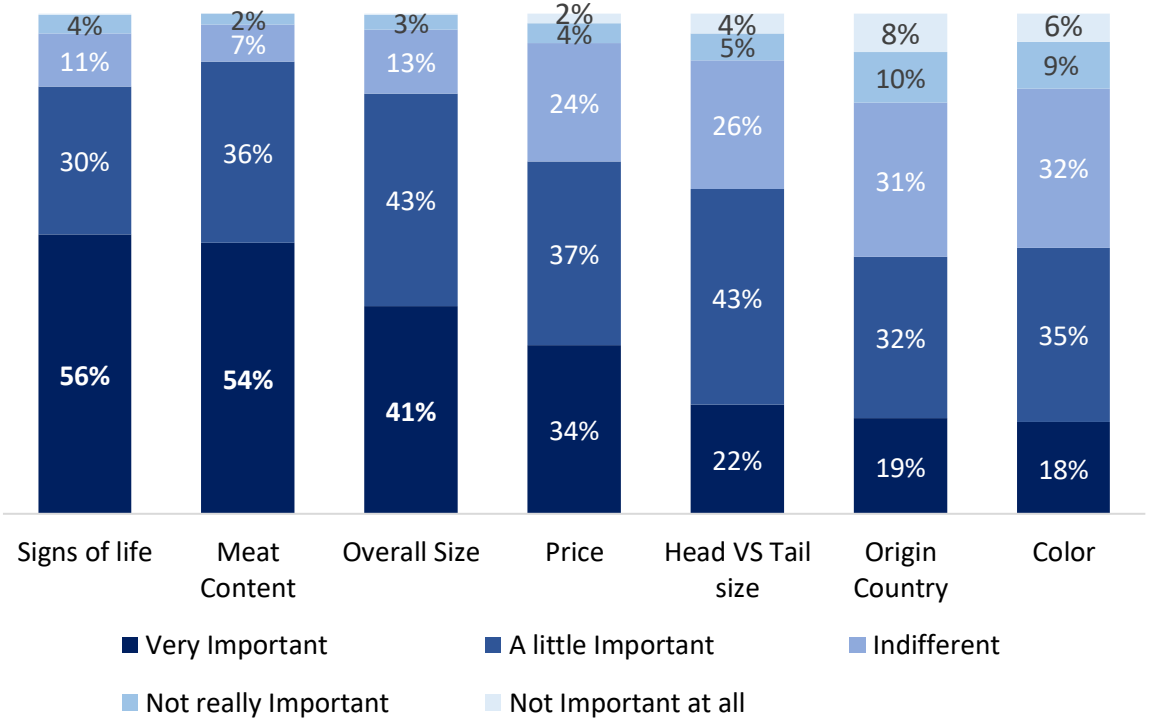
Majority of the respondents prefer to purchase medium-sized lobsters; Alive sign, meat content and overall size are the most important characteristics

Q3.2. What is your preferred size for purchasing a live Tropical Rock Lobster?



Most consumers prefer a lobster from 0.6 tob 1.4kg, no matter which country they come from. Compared with Chinese and Singaporean consumers, Korean consumers prefer a slightly larger lobster (≥1kg).

Q3.1. How important are the following characteristics to you when choosing and purchasing live tropical rock lobster?



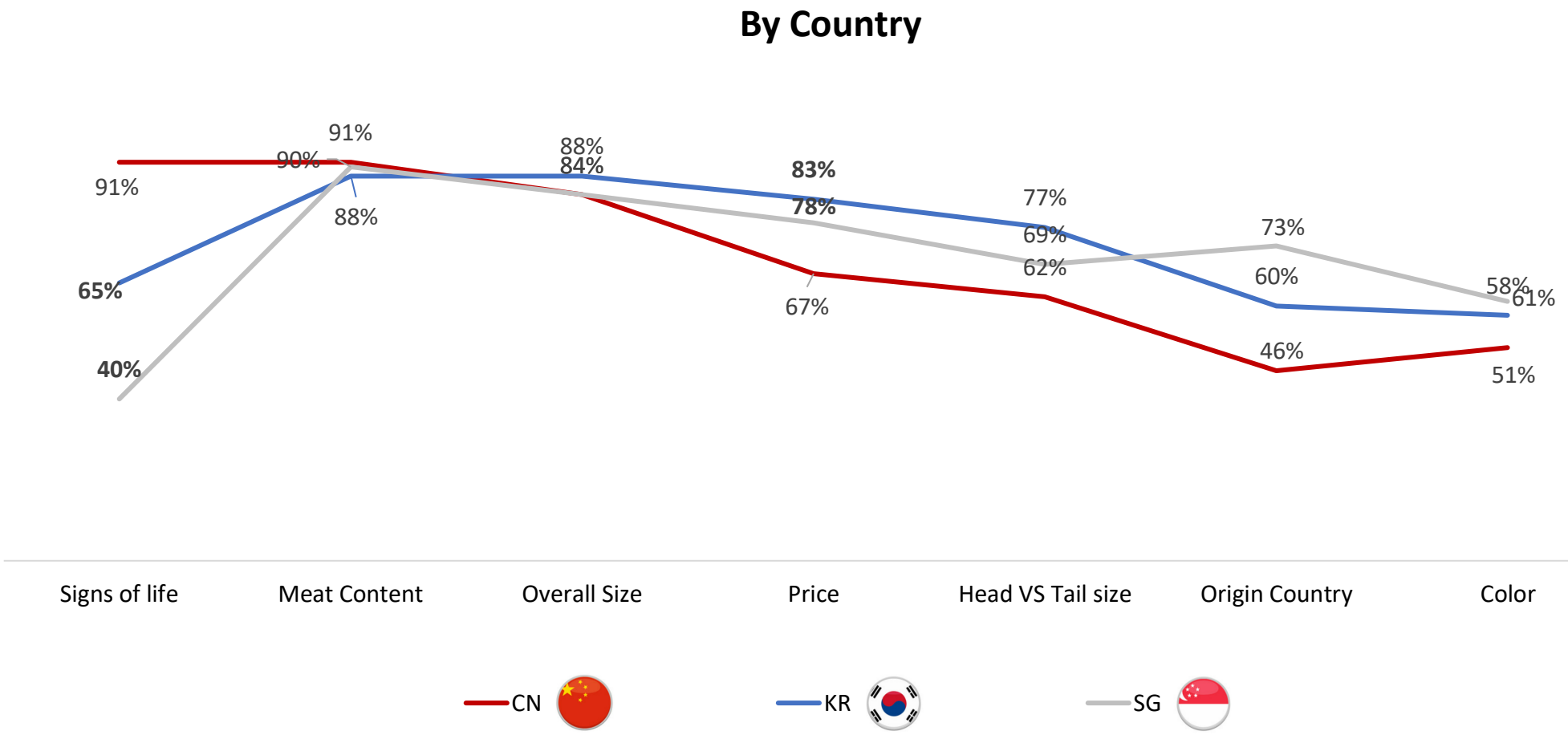
Signs of life, meat content and overall size are top 3 characteristics which consumers pay attention to when they make a purchasing decision. Color and the country of origin are perceived less important by them.

© Copyright 2023 – Daxue Consulting

Survey results - Decision-making criteria

Unlike Chinese consumers, Singaporean and Korean consumers consider price, head vs tail size as top criteria over sign of life. Still, meat content and overall size are top criteria across countries

Q3.1. How important are the following characteristics to you when choosing and purchasing live tropical rock lobster?

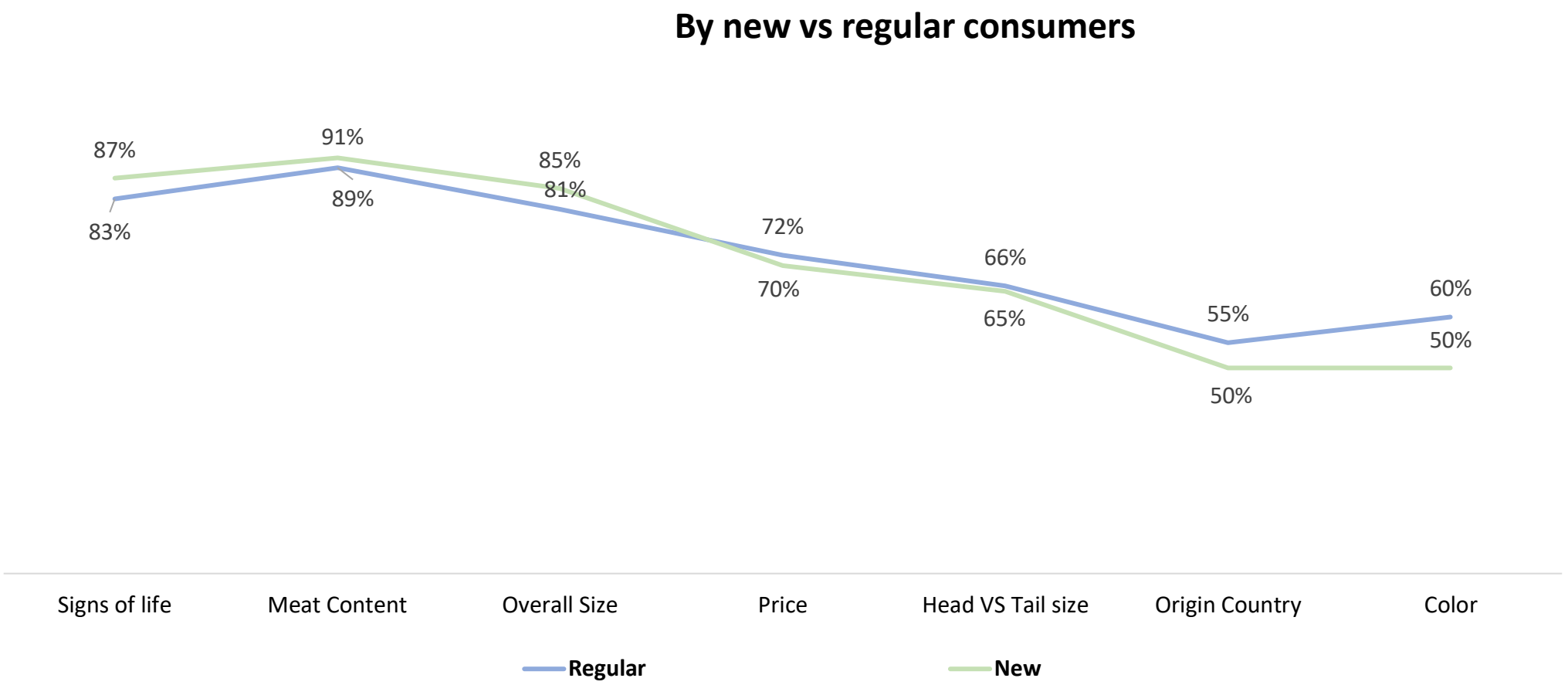


© Copyright 2023 – Daxue Consulting

Survey results - Decision-making criteria

The decision criteria are aligned between new and regular consumers, however the distribution of decision-making characteristics are slightly more equally spread among regular consumers

Q3.1. How important are the following characteristics to you when choosing and purchasing live tropical rock lobster?



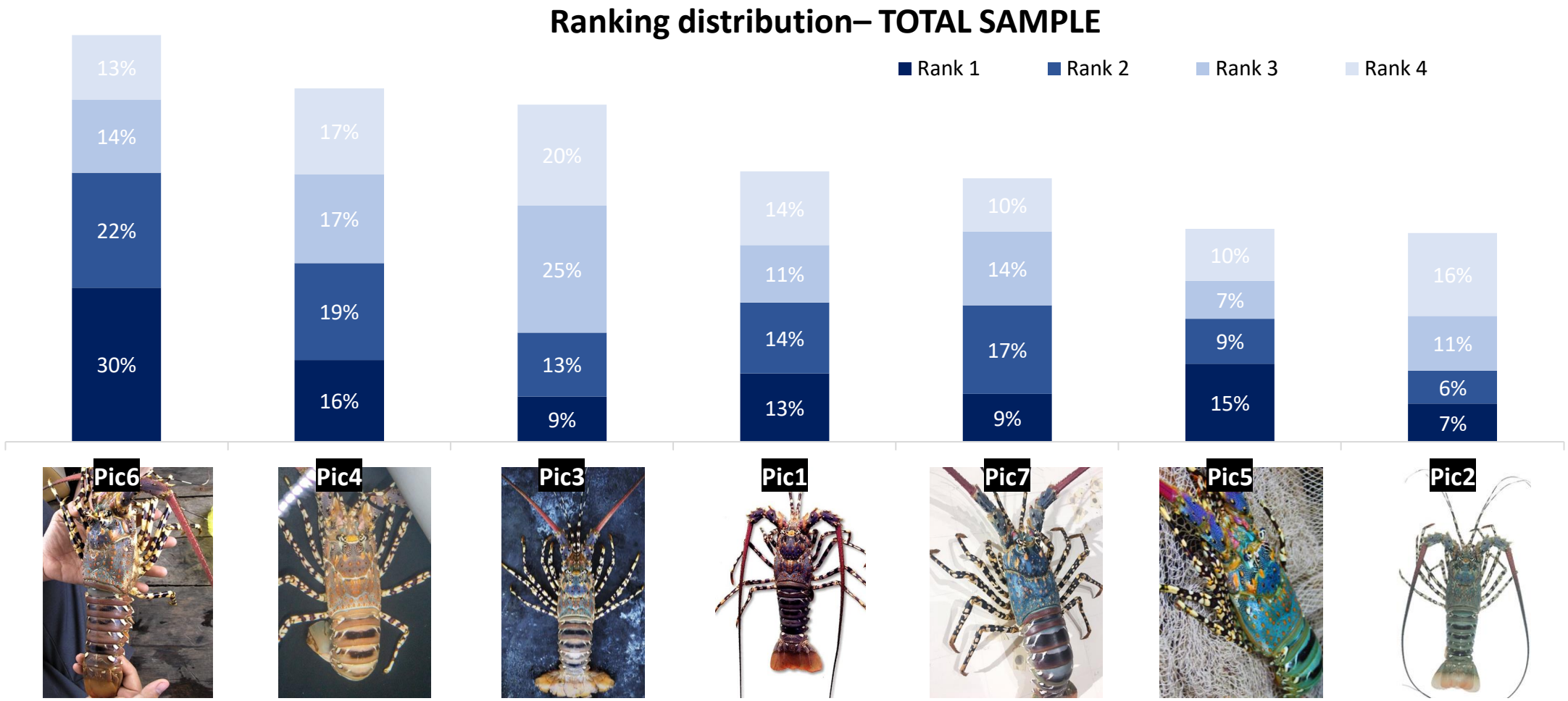
© Copyright 2023 – Daxue Consulting

Survey results - Picture selection

The picture 6 is getting the best score in both total mentions and T2B frequency

When aggregating the highest scoring pictures, it seems that respondents favor a plainer and *orangish* color, which we would associated with the most expected/typical lobster color

Q3.3. Below are 7 different images of oceanic grown tropical rock lobster. In terms of colour, please rank your top 4 from 1 to 4, where 1 is the most preferred.



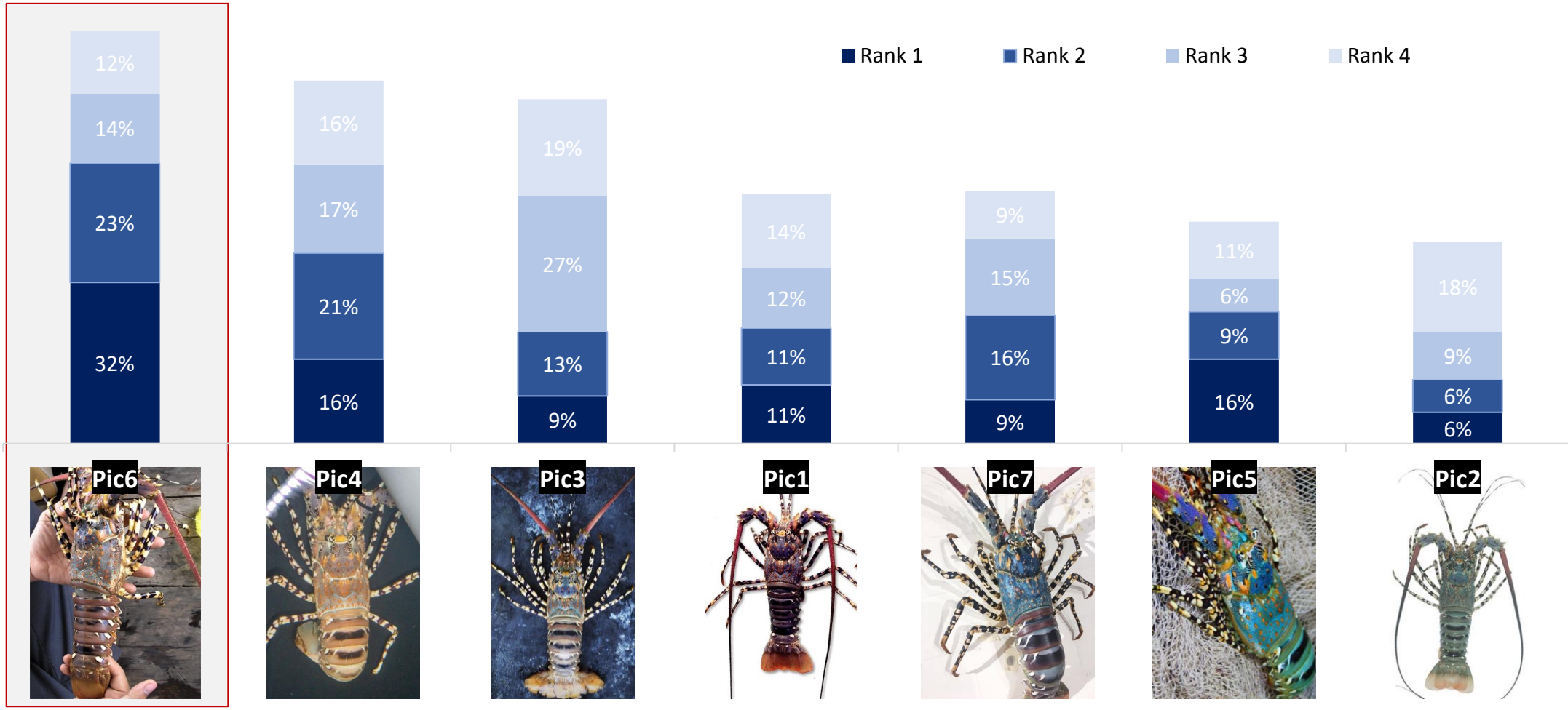
© Copyright 2023 – Daxue Consulting

Survey results - Picture selection

The Chinese respondents are driving the trend of the total sample, and the picture 6 is mentioned in the T2B answers by more than half of respondents

Q3.3. Below are 7 different images of oceanic grown tropical rock lobster. In terms of colour, please rank your top 4 from 1 to 4, where 1 is the most preferred.

Ranking distribution– CHINA



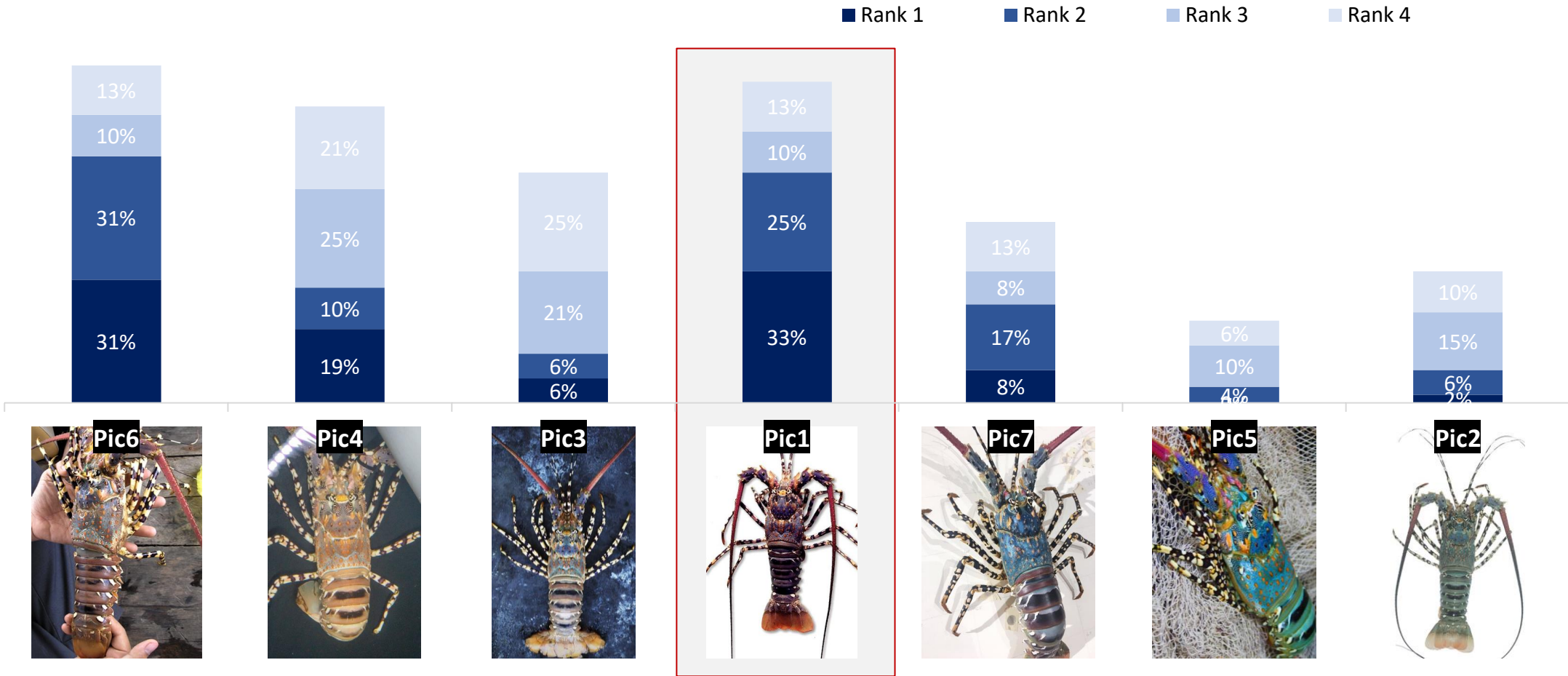
© Copyright 2023 – Daxue Consulting

Survey results - Picture selection

In Korea, while the picture 6 is still popular, the picture 1 is over-represented among the T2B answers clearly positioning as a favorite. Overall they favor the most plain colors.

Q3.3. Below are 7 different images of oceanic grown tropical rock lobster. In terms of colour, please rank your top 4 from 1 to 4, where 1 is the most preferred.

Ranking distribution– KOREA



© Copyright 2023 – Daxue Consulting

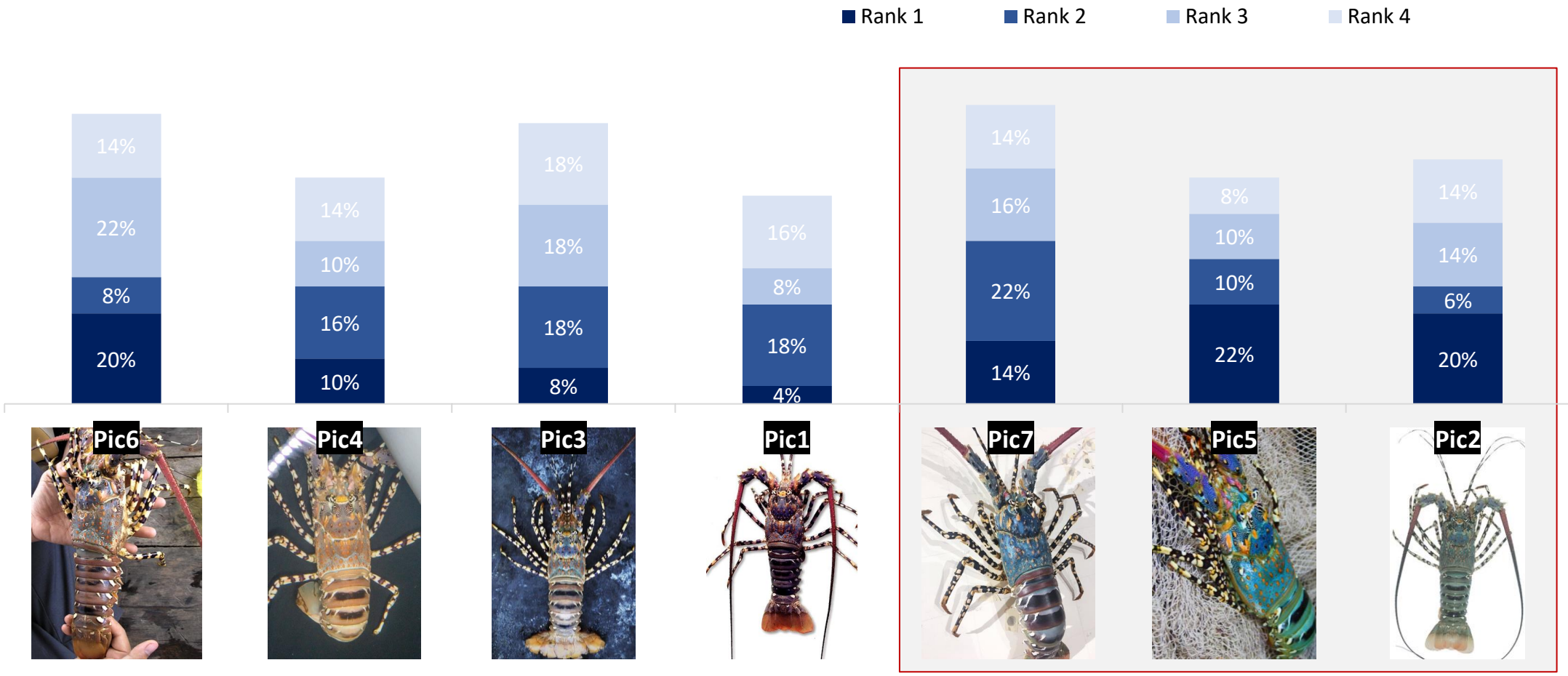
Survey results - Picture selection

In Singapore, the distribution is much more equal among pictures, with respondents seeminlgly less relunctant to select a more colored and unexpected lobster aspect

The pictures of the 3 most colored lobsters are actually collecting more than half of the top 1 answer among Singaporean respondents

Q3.3. Below are 7 different images of oceanic grown tropical rock lobster. In terms of colour, please rank your top 4 from 1 to 4, where 1 is the most preferred.

Ranking distribution – SINGAPORE



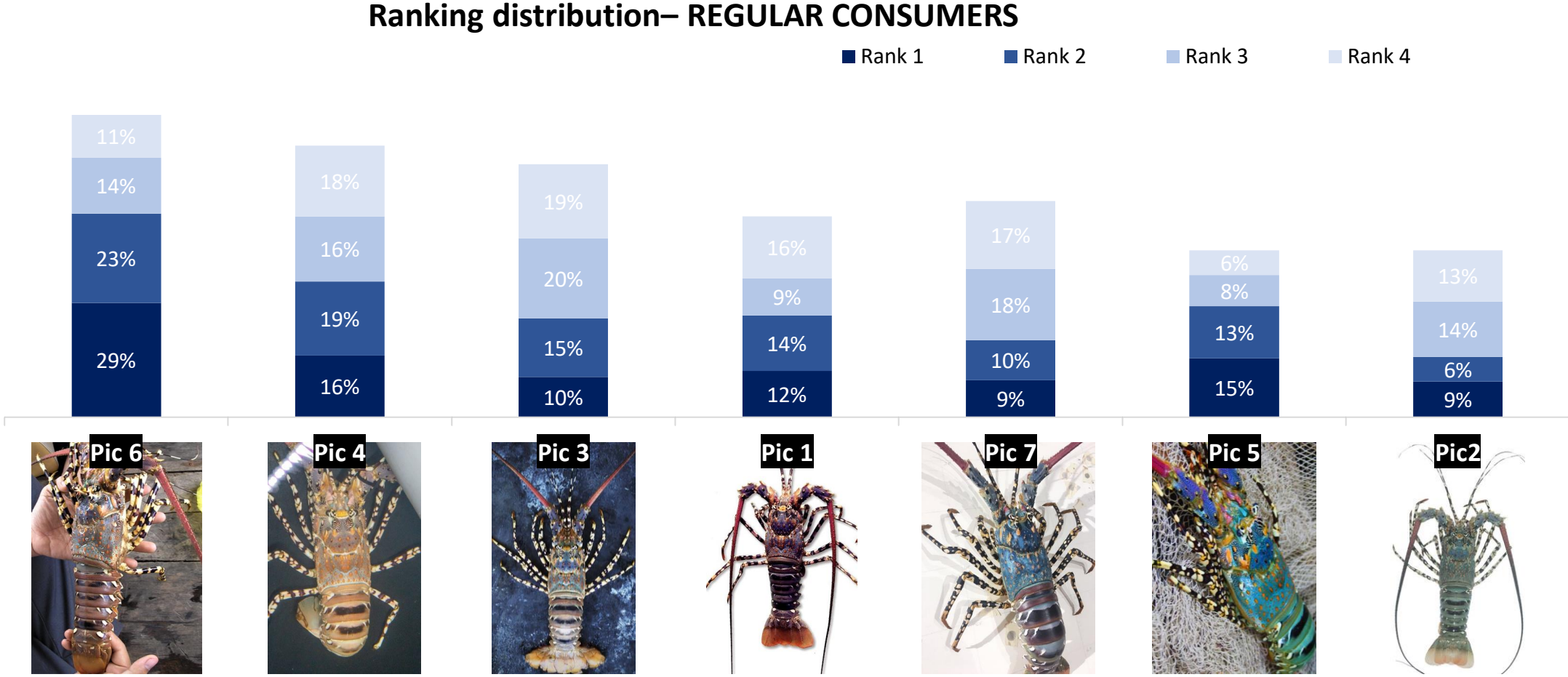
© Copyright 2023 – Daxue Consulting

Survey results - Picture selection

While the preferences of regular consumers follow the trend of the total sample, the gap between options is more narrow

As for Singaporean, these respondents are seemingly less reluctant to choose the most colored options (which we would associate with a higher level of product education)

Q3.3. Below are 7 different images of oceanic grown tropical rock lobster. In terms of colour, please rank your top 4 from 1 to 4, where 1 is the most preferred.



© Copyright 2023 – Daxue Consulting

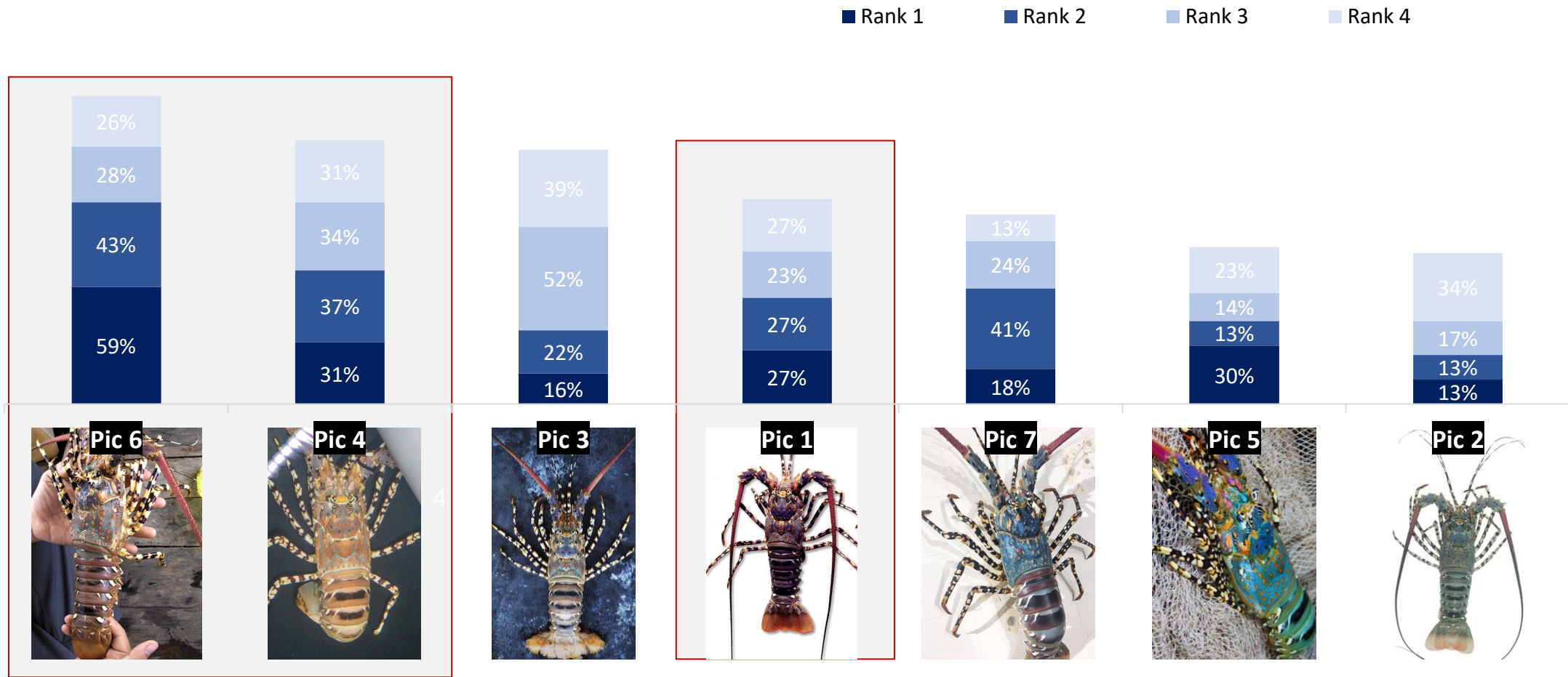
Survey results - Picture selection

To the opposite, new consumers, less familiar with the products, are gravitating more toward plain and expected lobster colors

Which we would associate with a need for reinsurance regarding product quality and safety when facing a new consumption

Q3.3. Below are 7 different images of oceanic grown tropical rock lobster. In terms of colour, please rank your top 4 from 1 to 4, where 1 is the most preferred.

Ranking distribution– NEW CONSUMERS



© Copyright 2023 – Daxue Consulting

Survey results - Picture selection

Summary: the picture 6 is by far the highest performing one, ranking as a top 2 options in any of the segment, and often being first with a sizable gap over other options

1

PICTURE 6

2

PICTURE 4

PICTURE 1

Other pictures that follow the same color patterns are also scoring well, providing a more **expected and typical visual for a lobster.**

They are especially **popular toward new consumers and respondents in Korea.**

3

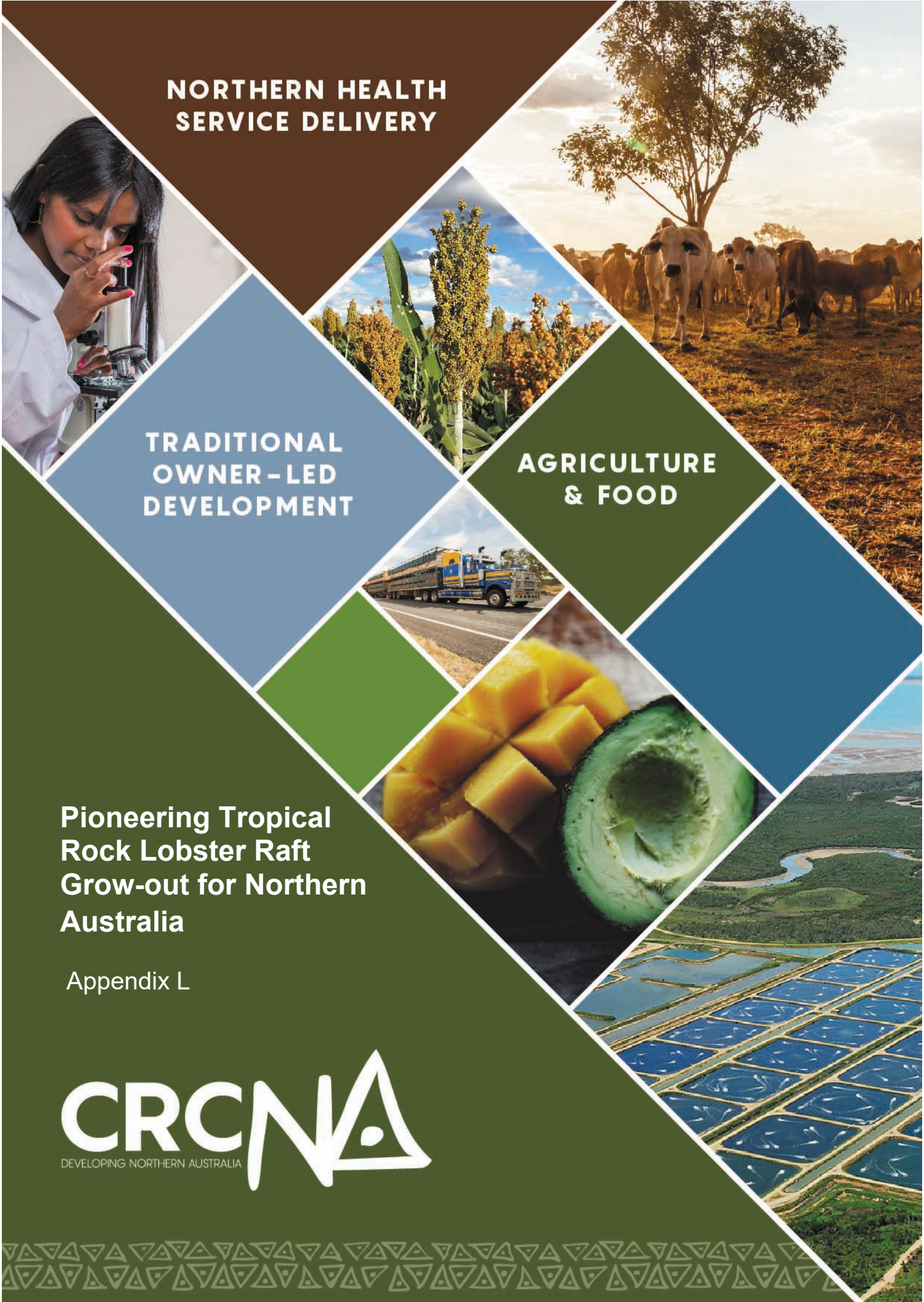
PICTURE 3

PICTURE 7

PICTURE 5

Pictures that are showing lobsters with more atypical and colorful patterns are scoring less among the full panel.

Still, the cross-analysis per segment shows that they can be **attractive to more familiar consumers.** It seems that **respondents in Singapore are also more favorable to them** than Korean and China.



Pioneering Tropical Rock Lobster Raft Grow-out for Northern Australia

Appendix L

CRCNA
DEVELOPING NORTHERN AUSTRALIA

Honey & Fox

Provenance Technologies Report

JULY 2023

DISCLAIMER

Created by Honey & Fox Pty Ltd for Ornatas as part of a project funded by the Cooperative Research Centre for Developing Northern Australia (CRCNA).

The information in this publication is intended for general use to assist Ornatas in choosing appropriate provenance technology to use for their lobsters. You must not rely on any information contained within this publication without taking specialist advice relevant to your particular circumstances.

While all reasonable care has been taken in preparing this publication to ensure that information is true and correct, Honey & Fox Pty Ltd gives no assurances to the accuracy of any information in this publication.

This publication is copyright. Apart from any use as permitted under the *Copyright Act 1968*, all other rights are reserved. However, wide dissemination is encouraged. Requests concerning reproduction and rights should be addressed to Ornatas Pty Ltd.

Honey & Fox

ornatas

The authors acknowledge the financial support of the Cooperative Research Centre for Developing Northern Australia which is part of the Australian Government's Cooperative Research Centre Program (CRCP), and the Fisheries Research and Development Corporation (FRDC). The CRCNA also acknowledges the financial and in-kind support of the project participants.

CRCNA

FRDC

www.honeyandfox.com.au

CONTENTS

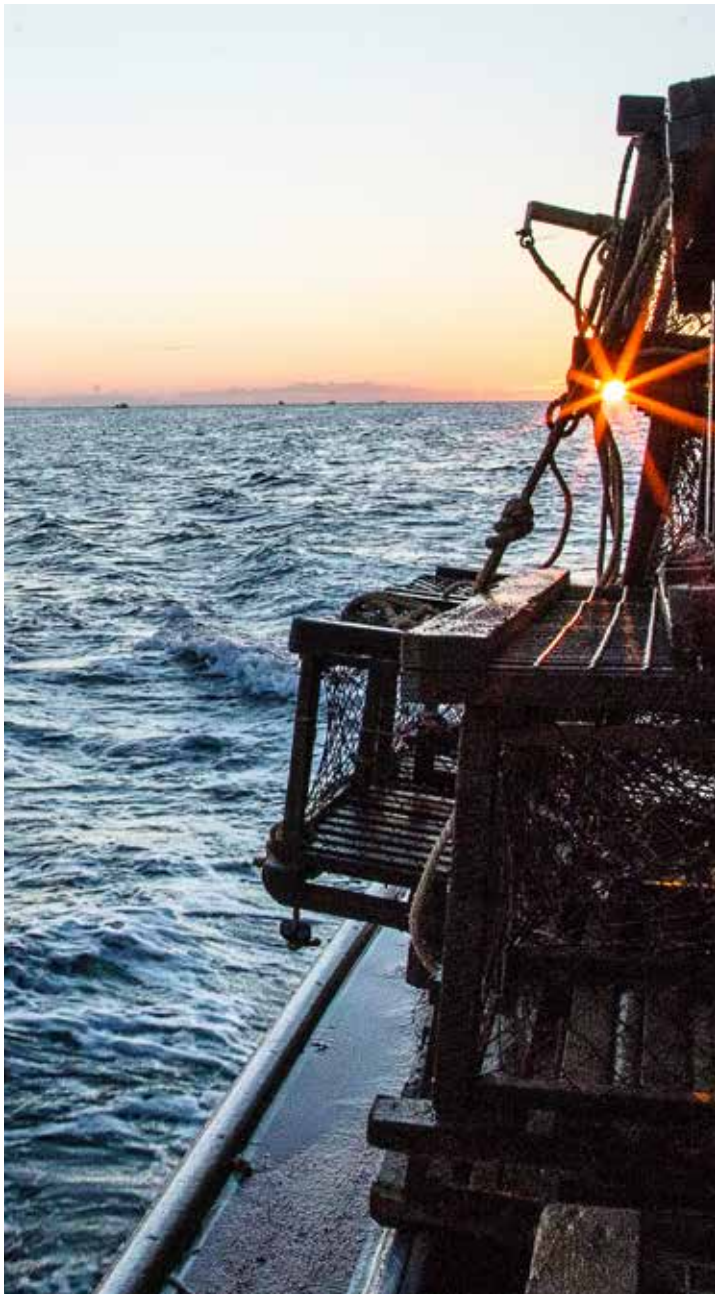
INTRODUCTION

Increasing consumers' physical and psychological distance from the source of food drives the growth in demand for food provenance. Consumers want to know where the food they eat comes from, who is producing it and how it is produced.

Fortunately, Australia has arguably the strictest food and farming regulations in the world, all to make food and drink the cleanest and safest available. Even so, there is an increasing interest in food provenance and provenance assurance from both consumers and government regulators. Both seek assurances that food is safe and, in the case of premium products, that it is authentic - "it is what it says it is".

Using storytelling to wrap information into a provenance story that transports people and provokes an emotional response is a powerful way to communicate with consumers. The impact of provenance stories can be amplified and multiplied by using technologies and platforms that prove authenticity and invite the audience to engage with and become a part of the story themselves.

The following pages introduce the concepts of provenance and authenticity and explore some of the many channels and technology platforms available that Ornatas could use to communicate its provenance stories to consumers.



PROVENANCE

Provenance relates to the origin of a product. It is about understanding its history, the story of where and how it came to be, and the journey it has taken to reach the consumer.

By communicating the provenance of produce and value-added products, including how it was produced and transported, farmers and producers may obtain a competitive edge over their rivals and, potentially, the ability to access niche markets and higher profits that might normally be unattainable (Wright, R, 2019).

The regional origin or 'provenance' values of products are generally communicated to consumers through branding and signs; these include geographical identifiers (place names), registered trademarks and registered geographical indications of origin
(W Caenegem, J Cleary, L Treguier 2016).

Various technologies are available to help communicate throughout the supply chain, increasing consumer knowledge of a product's provenance and enabling producers to differentiate themself in competitive markets effectively.

prov•e•nance

The place of origin of something

(Source: Cambridge Dictionary)

Key elements of provenance

Origins and history
Of the product and producers

Where and how
It came to be and is produced

Journey
It takes to reach the consumer

AUTHENTICITY

Authenticity has overtaken quality as the main purchasing criterion, just as quality overtook cost, and cost overtook availability before that. Put simply; authenticity creates value and benefits for customers.

Traceability and authenticity are often used interchangeably, but the two terms are distinctly different:

- Traceability involves record keeping that enables tracking an item (i.e. food) through all stages of production, harvest, processing and distribution.
- Authenticity (or genuineness) is more of a subjective evaluation of a product or brand by consumers (Napoli, J., Dickinson, S., Beverland, M.

Consumers expect the provenance story to be backed up with authenticity, which simply means the product is genuine - it is "as described".

It appears that the more virtual consumers' lives get, the more something genuine is desired. Modern consumers demand products that reflect this renewed desire for what is authentic.

au·then·tic

The quality of being real or true

(Source: Cambridge Dictionary)

Communicating authenticity

Creating and implementing authentic communication strategies work because they can:

- Elevate a business above the competition
- Build a business identity into something influencial
- Give substance to a business, its services and products
- Enable people to relate to a business
- Help people understand what offer is of benefit to them
- Tell people that what a business offers is of high quality
- Demonstrate a business is reliable and trustworthy
- Encourage engagement and can turn audiences into advocates

(Morhart, F., Malar, L., Guevremont, A., Girardin, F. & Grohmann, B. (2014) p. 200)

BUSINESS DRIVERS FOR PROVENANCE TECHNOLOGY

The major drivers for a provenance, authenticity and traceability system include:

- Help comply with food safety and QA requirements
- Help manage and monitor the performance of the supply chain (eg. to ensure temperature compliance)
- Track where products are during its journey to the customer
- Prove and share product provenance stories
- Make it easier for customers and consumers to know whether they have a genuine product
- Reduce opportunites for your products to be copied or for the packaging to be reused for other products
- Help verify your product claims (eg. provenance, organic, sustainability, quality certifications)

Food Safety and QA

Legally, any food business in Australia must comply with the Australian Food Standards Code, which requires tracing “one up and one down” and keeping records that enable recalls to happen quickly and efficiently. Automating this process saves time and money and potentially avoids risking your brand reputation.

Provenance technology can be integrated with food safety and QA systems, and, depending on the system, can demonstrate compliance with those requirements.

Manage and Monitor the Supply Chain

Supply chain performance is often a critical part of product provenance. Provenance technologies can help monitor and record the condition of your products at any stage, from production to harvest, processing, and distribution.

In addition to assuring provenance, track and trace capabilities can improve business efficiency, helping to meet your regulatory obligations, manage your inventory, pinpoint waste and identify opportunities for process improvement.

Track Product During Journey

Trackers can provide information about a product's journey to reach a customer, with or without temperature and other monitoring data.

Some trackers can send the data back in real time, while others rely on data uploaded to a system after completing the journey. This may mean that you rely on your customer or representative to get the tracker and either upload it or send it back to you for uploading.

Proving Product Provenance

Technologies using DNA, trace elements, and chemical profiling can be used to link a product to a specific geographic location.

This capability can be combined with other technologies to help you tell the story of where and how your product was produced and the journey it takes to reach your customers.

Businesses that use provenance technologies together with a compelling story often get a competitive edge and potentially access high-priced niche markets.

Preventing Food Fraud

Globally, food fraud is rising and a significant challenge for businesses to manage. Product fraud deceives consumers by providing them with a different, often lower quality product against their knowledge.

Provenance technology, perhaps combined with other devices such as tamper-evident packaging or labels, can help minimise this risk.

Technology is not the only answer; you must educate your supply chain and customers about what to look for and how to tell a genuine product from a fake one.

Verifying Product Claims

Consumers increasingly seek external verification of product claims such as origin, organic, sustainability, and quality certification.

Even if you don't sell directly to consumers, your customers eg wholesalers, retailers etc, may also want documented proof of your claims. Some, but not all, provenance technologies can help with verification.

CHOOSING THE RIGHT PROVENANCE TECHNOLOGIES

Technologies for communicating product provenance stories are increasingly focusing on bringing the consumer closer to the source – aiming to reveal and restore relationships with the world that is somewhat alien and distant to urban lifestyles.

There are many systems and platforms to choose from, and more are being developed regularly. Primary considerations when selecting a system or a platform include:

- Level of investment required, both upfront and ongoing
- Ease of use by the business and the consumer
- The amount of information that can be stored and communicated through the chain
- How secure and trustworthy is the system
- How supportive the supply chain partners are

Start by making decisions about what and when you would like to track and trace your products:

What will you track?

Individual products or a batch of products, the choice is yours - Do what makes sense to you and your customer. A batch usually has products with similar attributes grouped together e.g. harvest location and date

How will you identify them (your unique identifiers)?

Work out how you will uniquely identify each batch or product (traceable entity). This can be as simple or as complex as you like.

What data do you want?

For example, you might be interested in where the product or batch is, the time spent in each location, the temperature of the product itself and/or the ambient temperature around it, how many times it has been scanned or handled etc.

Where do you want to track?

At the point of harvest?

When it arrives at the processing or packing shed?

When it leaves the processing or packing shed?

When it reaches the customer?

TECHNOLOGY OPTIONS

There are many provenance and authenticity platforms to choose from, with new technologies and platforms being introduced all the time. Some are specialised for specific products while others are more general. Some of the technologies that might be useful for Ornatas are detailed here.

Source Certain

Source Certain is an Australian company working internationally. The platform is underpinned by a robust, definitive, tested and validated scientific method for establishing a product's provenance.

It is used to determine a product's chemical profile, which reflects the geographical location where it was grown and/or the system by which it was produced.

www.sourcecertain.com/service

The IBM Food Trust

Built on blockchain, IBM Food Trust is a collaborative network of growers, processors, wholesalers, distributors, manufacturers, retailers and others. A system that works internationally, the platform connects participants through a permissioned, immutable and shared record of food provenance, transaction data, and processing details. Visibility and accountability are enhanced across the food supply chain.

The focus is on enabling data sharing between trusted participants, traceability beyond the one-up, one-down and underpinning certifications.

There are plans for different types and sizes of businesses.

www.ibm.com/auen/marketplace/food-trust

Fresh Supply Co

Fresh Supply Co is an Australian marketing technology company for fresh food products. The company works collaboratively with producers to identify how products can be tracked without disrupting operations. Data that is captured is used to create a full narrative of each unique product unit that's tracked. The blockchain-based, track-and-trace platform is integrated with content development (e.g. recipes) to support storytelling on a range of platforms. The company also helps producers establish performance metrics and reporting.

www.freshsupplyco.com

Provenance

Provenance, a UK-based company operating worldwide. The Provenance platform is underpinned by blockchain and open data. Provenance is a platform for businesses and shoppers to provide greater transparency about their products and the journeys to the customer.

Provenance enables businesses to share stories and verifiable claims about themselves and their products in a trustworthy way. It can be taken further by showing the traceability of each batch or item through its tracking tool.

This creates a time-lined supply chain with product data unique to each batch. The transparency framework is a structured suite of product 'claims' with proof provided via a trust engine that links to third-party data sources.

The Provenance publishing suite enables the transparency story to be shared directly with customers. Whether it is via publishing on a website, as a standalone URL, or as 'cards' to use across social channels, content is optimised for any device or platform. It can be tailored for all points of the customer journey.

For Provenance's case studies and company information on those using the platform go to www.provenance.org

Trust Provenance

T-Provenance Pty Ltd (Trust Provenance) is an Australian start-up that works to build a new level of trust and quality management into agricultural supply chains. It does this by bringing farmers, logistics companies and distributors together on a blockchain platform driven by autonomous Internet of Things (IoT) measurements. The platform supports previously impossible collaborations and efficiencies by identifying, measuring and analysing supply chain

blind spots, resulting in new levels of quality assurance, waste reduction and supply chain efficiency gains.

The Trust Provenance blockchain platform is agnostic for produce type; that is, it works for fruit, vegetables, meat, wine, seafood etc. It creates the trust environment for data integration and information exchange.

Collaboration on quality assurance practices is automated and verified inchain to guarantee quality, reduce wastage, and streamline exception reporting and reconciliation between stakeholders. Trust Provenance replaces the proof and recourse cycle with trust.

www.trustprovenance.com/

Two Hands

Two Hands is a start-up company that aims to connect fishers and farmers with high-end restaurants underpinned by guarantees of provenance using blockchain.

For more information, go to

www.2hs.info/

Manbullo Mangoes

CASE STUDY

BACKGROUND

Trust Provenance, in collaboration with Manbulloo, Growcom, and the CRC for Developing Northern Australia, commenced a smart-supply-chain traceability project in 2017, with a focus on providing real-time and secure information on all mangoes from the paddock to the retailer, to optimise the journey, identify pain points and ultimately provide better quality fruit to consumers.

As the mangoes go from point-A to point-B, data is collected into separate systems by each stakeholder in the supply chain. Trust Provenance's software collects data from each of these systems.

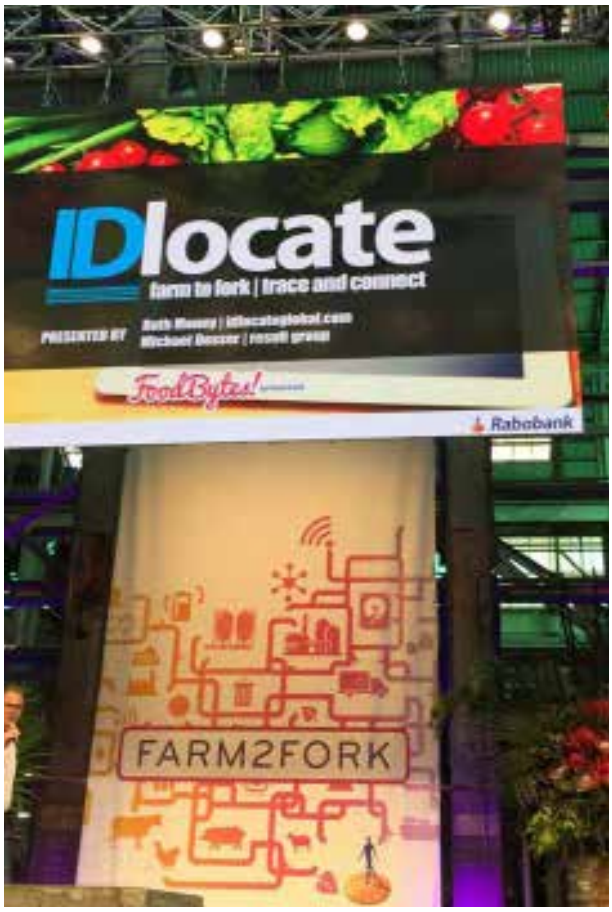
IMPACT

Scott Ledger from Manbullo provides an overview of the key outcomes of this collaboration.

- Saving time and access to data
- One Platform
- Customer Value
- Efficiencies
- Value Proposition and ROI

Trust Provenance

Read full case study here:
https://www.crcna.com.au/resources/publications/smart-supply-chainsproject-fact-sheet



IDlocate

The NZ-based IDlocate Authenticity Platform assists brands to create connections with global consumers to prove provenance and authenticity anywhere, anytime. IDlocate's anticounterfeit logic provides customers with a brand-verified purchase, using unique QR codes that bring authenticity and provenance stories to life directly from the product packaging.

Using the combination of unique QR codes and the IDlogic fraud engine, a series of checks are activated as each scan occurs. These checks ensure the product is legitimate, in the right market and alerts are generated when there is an inconsistency.

www.idlocate.co.nz

Laava Smart Fingerprints

A step up from the QR code is Laava Smart Fingerprint®. QR codes were designed to identify, not authenticate, and are inherently insecure. Laava Smart Fingerprint® technology has removed the data from the code and turned it into a secure, scannable mark: as unique as your fingerprint.

Fingerprints are scanned by customers using their smartphones to receive a verification of product authenticity and connect them with the provenance story. The fingerprint can link to other traceability systems such as trackers, trace elements and blockchains to provide an end-to-end authenticated product provenance story.

https://laava.id/

MOWI Salmon

CASE STUDY

BACKGROUND

The world's largest producer of Atlantic salmon chose EVRYTHNG to help launch its brand with 100% transparency, powering food traceability and provenance for its product lines. Now, consumers can trace the full lifecycle of the salmon they're about to purchase. With this transparency, Mowi is projected to increase sales through brand loyalty.

"We like to think of ourselves as leaders when it comes to food safety and sustainability, and EVRYTHNG helped us showcase that to consumers all around the world." Ola Brattvoll Chief Operating Officer, Sales & Marketing at Mowi

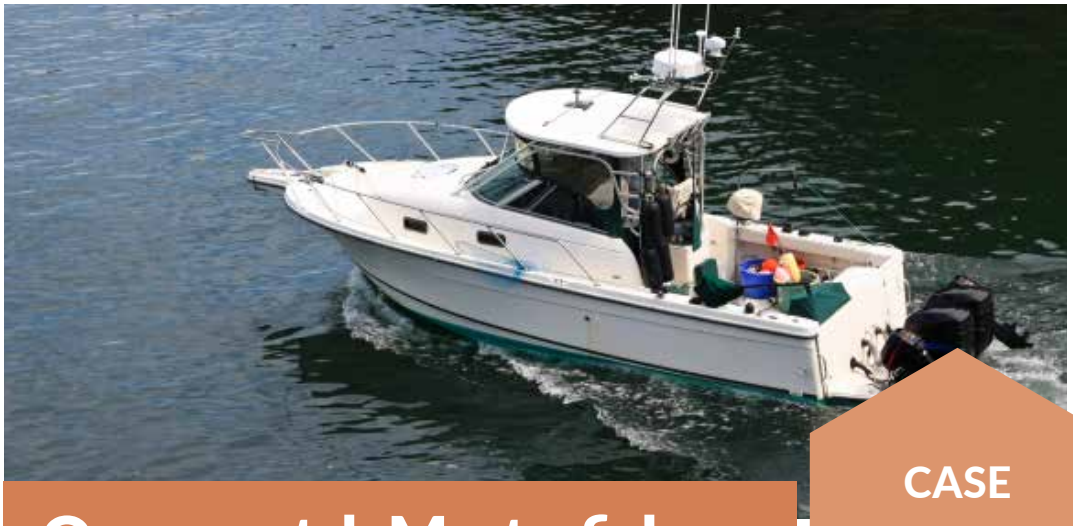
IMPACT

Mowi aims to differentiate its product in the eyes of consumers by highlighting its superior quality while giving consumers the transparency they crave.

Mowi also aims to build trust and ultimately grow sales by forging a digital connection with consumers.

Mowi intends to capture valuable insights from widespread consumer engagement, where previously, it had no visibility. By understanding where, when and how consumers engage, Mowi will gain greater insight into what consumers want and use that knowledge to inform future marketing programs.

Read full case study here:
https://www.crcna.com.au/resources/publications/smart-supply-chainsproject-fact-sheet



Oceanwatch Masterfishers

CASE STUDY

BACKGROUND

The OceanWatch Master Fisherman program is a formal training and assessment program for professional fishers to recognise those in the industry that is continuing to raise the standard of responsible fishing in Australia.

Once accredited as an OceanWatch Master Fisherman, individual stories of the fishers are published on the OceanWatch website as part of a “meet your fishers” series. Each story describes the fisher’s fishing location, what they catch and why they care, and a photo of the fisher. Stories may be published as text content or video. A QR code is provided to the fisher to put on

to their products and marketing materials to link the consumer back to the source

IMPACT

Stories of each individual fisher demonstrate to the consumer and their community that they are personally committed to responsible and sustainable individual fishing practices, going above and beyond the requirements prescribed by state, national and international regulations.

Read more: https://oceanwatchmasterfisherman.org.au/

Reid Fruits

CASE STUDY

BACKGROUND

Reid Fruits had been searching for a traceability solution to help achieve its product integrity objectives while also helping to tell its brand story. The solution needed to be at a "commercially relevant price point".So they teamed up with product integrity start-up Laava to prevent counterfeiters from copying Reid's distinctive packaging.

Unlike bar codes or QR codes, Laava's smart fingerprint technology is much harder to impersonate or replicate and much more secure, making it more resistant to counterfeiting. It also delivers detailed brand and product information and interactive experiences to customers.

IMPACT

- 10 counterfeit attempts on Reid Fruits' cherry boxes were foiled in China in cherry season 2019–20.
- 4,470 Laava Smart Fingerprints scanned on Reid Fruits cherry boxes
- A 2.9% scan rate with a 15–20% engagement level, with only a limited campaign to drive awareness.

Since the first season (2019-2020), Reid Fruits have added functionality to their traceability system. They are now automating product identifiers and tracing their product through the supply chain to monitor performance.

Read more: https://laava.id/reid-fruits/

NEW AND EMERGING TECHNOLOGY

These technologies and platforms aren't commercially available, but they're on the horizon.

Smart Dust

Smart dust is a network of nanotechnology that can permeate different environments, be used to collect and communicate information, and then act on it. Key components driving the development of smart dust are nanoscale sensors and robots, nanoscale power generation and storage devices, and molecular machines. Applications can include tracking products from producer to consumer.

While it has been around for quite a while, there are still concerns about its use that need to be overcome. These include privacy concerns (the particles are so small they are difficult to detect), control (retrieving the devices once they are deployed) and cost. As with any new technology, these issues will be resolved as application, many of which are only in the concept stage, and use increases.

Context-aware Computing

Context aware computing is where devices (such as smartphones, laptops and tablets) can detect who's using them, what they're doing, when they're doing it and where they're doing it. This information can then be used to better target products and services to consumers. An example of context-aware computing is the screen turning when you move your phone. More and more sophisticated applications are being developed so we can expect these will include applications used to target product provenance stories and information to consumers.

Lobster fingerprint technology

Current lobster identification uses plastic tags attached to antennae, which can be intrusive and requires re-tagging after each moult. Tags are then manually re-assigned by using the pattern between the horns. This requires additional labour and introduces the risk of mislabelling.

Ryder Jamson conducted an honours project at IMAS on Image Recognition to Fingerprint Individual Adult Tropical Rock Lobster.

The proposed idea is to use a smartphone app that which would allow consumers to scan the pattern on a lobster and identify its provenance in real-time. In addition to the app, there is potential for an in-tank monitoring system which could display labelled individuals in real time and track the movements of the lobsters over time.

Neither the app nor the in-tank monitoring system have been made commercially available, but it is important to keep track of how this project may develop as there is a possibility this could be of great benefit to Ornatas.

Source: Ryder Jamson IMAS PhD Research on Lobster Fingerprint

Blockchain is typically the backbone of modern traceability and authenticity for systems and platforms, because it's highly secure. Blockchain is literally digital information ('blocks') stored in a database ('chains').

Appendix 1
Blockchain, QR Codes and Smart Labels Explained

BLOCKCHAIN

The goal of blockchain is to allow digital information to be recorded and distributed, but not edited. Blockchain works via the following processes. The block has three types of digital information:

1. Transaction information, e.g. time, date, amount paid.
2. Who's participating in the transactions using a unique digital signature.
3. Information that distinguishes each block from each other. This is a unique, identifying code called a 'hash'.

The chain consists of multiple blocks joined together.

There are four things that must happen for a block to be added to the chain:

1. A transaction must occur.
2. The transaction must be verified by a network of computers. These networks confirm the details of the transaction.
3. After the transaction has been verified, all the information about that transaction is stored in a block.
4. The block is then given a hash. The block is also given the hash of the previous block added to the blockchain. The block can then be added to the blockchain.

A copy of the blockchain is then placed on every computer in the network (this can be thousands or even millions, such as in the case of cryptocurrency).

Each copy of the blockchain is identical and spreading that information across a network of computers makes the information more difficult to manipulate. As such, a hacker would need to manipulate every copy of the blockchain on the network.

New blocks are always added to the 'end' of the blockchain. Once a block is added to the blockchain, it becomes very difficult to edit and impossible to delete. This is because each block has its own hash code and the hash code of the block before it. If the data is changed in one block, then a new hash code is generated again, making it difficult, if not impossible, for a hacker as all of the data in all of the blocks would need to be changed.

QR CODES

A QR code (short for 'quick response' code) is a type of barcode that contains a matrix of dots. It can be scanned using a QR scanner or a smartphone with a built-in camera.

QR codes are particularly popular in China as the popular WeChat app uses QR codes to link people with each other and with brands. While they are not as popular in Australia, QR codes are a powerful platform for product provenance storytelling. Scanning a QR code takes on average 15 seconds. This includes the time it takes for the consumer or interested person to take out the smartphone, open a scanning app, hold the device steady towards the code and scan.

QR codes have a high information storage capability, including text, URLs and webpages. QR codes have a tolerance of up to 30% damage without impeding their ability to be used effectively. The food and beverage industry has adopted the QR code internationally due to its ability to link consumers to the product authentication information. They are also easy to generate and link to information. They can also be printed on various materials, including waterproof packaging and labels.

QR codes can be used to disseminate information to the consumer including:

- Offering product information and specifications
- Delivering coupons and relevant deals
- Boosting app downloads
- Delivering product videos
- Increasing post-purchase engagement

SMART LABELS

An umbrella term for any labelling or coding that uses technology to add functionality and data beyond a traditional simple barcode, for example, data-embedded barcodes, RFID, and QR codes.

Radio Frequency Identification Data (RFID)

It uses small tags attached to products to store and transmit electronic product codes (EPC). Passive RFID tags require stationary or handheld readers that electronically prompt the tags to share data. Unlike barcodes, RFID tags do not need to be in the line of sight of a reader. Active RFID tags use their power supplies to send information to readers that can be up to a mile away. Implementation of RFID requires tags, labelling devices, readers, and information technology systems.

Barcodes

A series of thin and thick lines that carry machine-readable information about a product: the barcoding standards for consumer products are EAN-8 (8 digits) and EAN-13 (13 digits). The standard used for logistical units is ITF 14 (14 digits). GS1-128 (up to 129 alpha-numeric characters) is used within the GS1 Standard to allow barcodes to include

specific product attributes such as harvest dates, harvest locations, lot numbers, quantities, weights, and packing dates. The hardware for barcode labelling includes labels, label printers, scanners and computer systems.

Dynamic QR Codes

Static QR codes have the disadvantage of not being able to be updated. You can create a dynamic code to keep from being stuck with a QR code that you can't update. With this type of code, you can change the target URL or content at any time, even after you've already printed and distributed hundreds of your marketing materials.

The key is to make sure that you connect your QR code to the right information that you want your customer to see. Imagine your customers' dismay when they take the time to scan your code to find a broken link or outdated information. This is a mishap that can be easily avoided.

It is a good idea to add a call to action that makes consumers want to scan your QR codes. Even a simple 'Scan Me!' has proven to engage a lot more users than codes without a call to action. The more interesting the call to action, the more likely consumers will scan your QR code. For example: 'scan this code to see our fishers and/or farmers in action!'

Honey & Fox

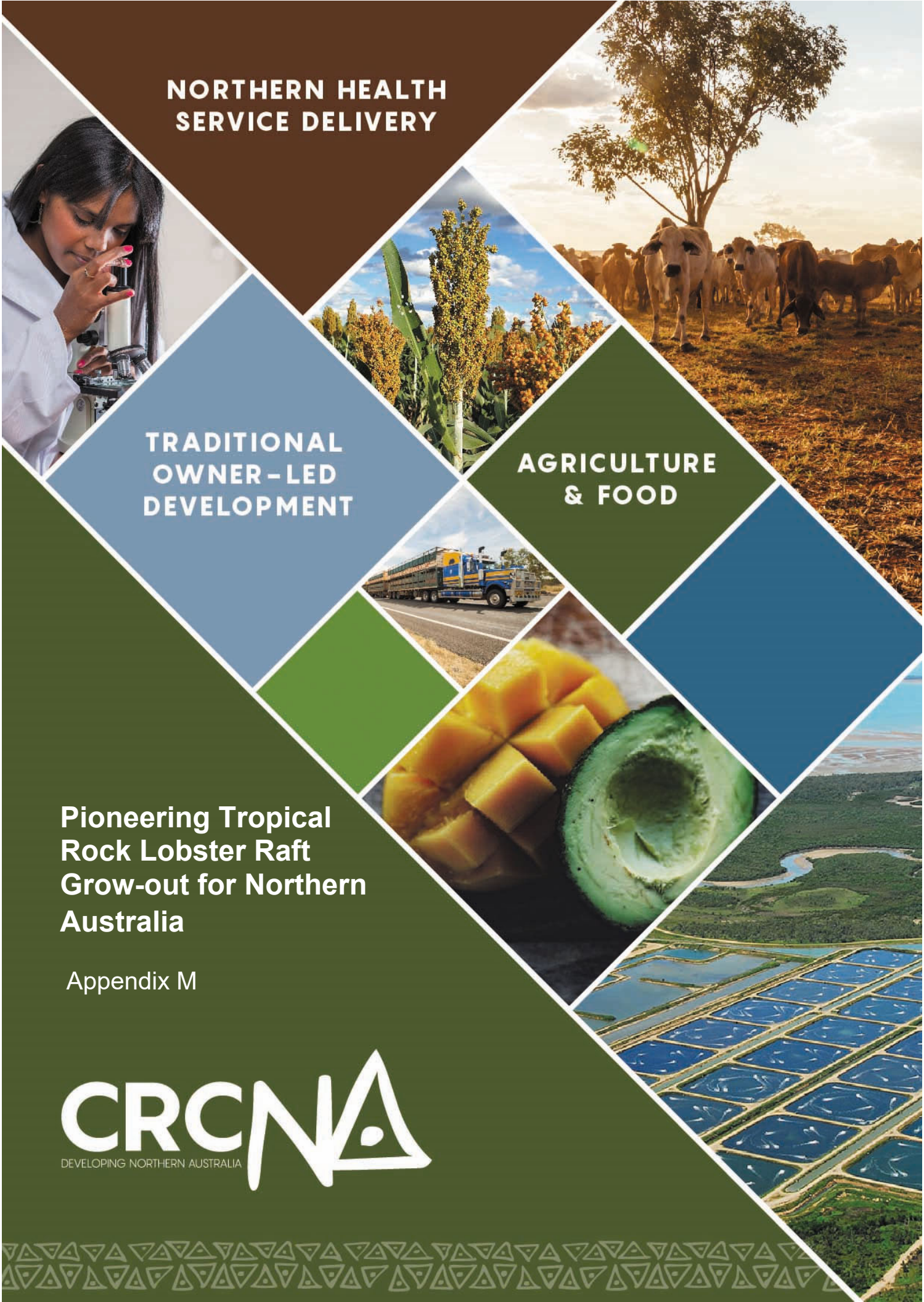
Report prepared by Honey & Fox Pty Ltd for Ornatas Pty Ltd

www.honeyandfox.com.au

The authors acknowledge the financial support of the Cooperative Research Centre for Developing Northern Australia which is part of the Australian Government's Cooperative Research Centre Program (CRCP), and the Fisheries Research and Development Corporation (FRDC). The CRCNA also acknowledges the financial and in-kind support of the project participants.

CRCNA

FRDC



Pioneering Tropical Rock Lobster Raft Grow-out for Northern Australia

Appendix M

CRCNA
DEVELOPING NORTHERN AUSTRALIA

Honey & Fox

Ornatas Farm Visit and Product Tasting Report

March 2024

Purpose

This document outlines the key findings and recommendations following a farm visit and product tasting undertaken by a select group of experienced Australia-based chefs, retailers, and wholesalers.

Aim

The farm tour and product tasting aimed to investigate further and explore some of the findings of earlier demand research in more detail. This included getting some user-generated language around describing the hatchery and sea-raft grow-out process and understanding the relative importance of different. quality parameters

Participants

The participants were selected based on their extensive experience as end users (Chefs, retailers, wholesalers) working with lobsters in Australia. It was a restricted group because we did not want to create any expectations about commercial availability and supply. The participants and their experience is summarised below. See attachment 1 for the full details.

Umar Nguyen
Umar, known as "the Fish Girl," is a food service market development expert. She connects producers directly to processing, logistics, and distribution expertise and designs and executes sales and marketing campaigns that deliver tangible benefits to all involved.

Umar's distinctive strategy involves connecting producers with chefs nationwide who interact with Australian seafood consumers daily, from pubs and clubs to fine dining establishments. This direct connection is pivotal in amplifying the reach of her efforts.

Jake Nicolson
Jake is currently the executive chef of the Ghanhem group incorporating diverse venues:

- Blackbird Bar and grill (steak and seafood)
- Blackbird events (Riverview events/function)
- Donna Chang (modern Chinese with live tanks in the restaurant)
- Boom Boom room (Japanese)
- Iris rooftop (Spanish)
- Bisou Bisou (French)
- Hotel X (hotel/ function)
- Modern Vietnamese opening in August

Nicholas Redsell
The executive chef from The Ville and the newly opened ArdoTownsville overseeing the operations of several venues including:

- Miss Song Modern Asian

- Quarterdeck – bar/bistro
- The Palm house – Tropical
- Splash Bar – Bar/ Bistro
- Spin Café – café
- Sports bar – bar
- Marmor
- Terasu
- Ardo Rooftop

Damien Gan, Custom Seafoods
Custom Seafood has supplied the hospitality and catering industry with some of Australia's finest seafood for the past 16 years. With over 40 years of combined experience, Damien and Grant deal with some of the world's best chefs and fishers. They wholesale live, fresh and frozen seafood.

Max and Francoise Pantachini, Preston Fresh Seafood, Cairns
Max and Francoise established retail and wholesale operations in Cairns in 1993. Preston Fresh is now the largest Seafood Retail Shop in Cairns. They bring a large component of their hospitality background (including providing seafood to major VIPs like QE2) into their work, having great respect for their produce and a passion for food creation.

Survey and in-depth interviews.

All participants were asked to complete a short survey about their quality expectations of lobster generally. They were then interviewed to document their impressions of the Ornatas hatchery raised sea-raft grown product. (See Attachment 2). The sample size was small, so we cannot extrapolate the findings. We have commented on whether the findings are consistent with previous research.

Survey findings

All respondents (n=6) had had experience with a variety of lobster species, one person had not had any experience with Tropical Rock Lobster, and some had had experience in Australia and overseas. Participants were asked about their preferences in terms of product format (Fig 1) and size (Fig 2)

Figure 1: Preferred product formats

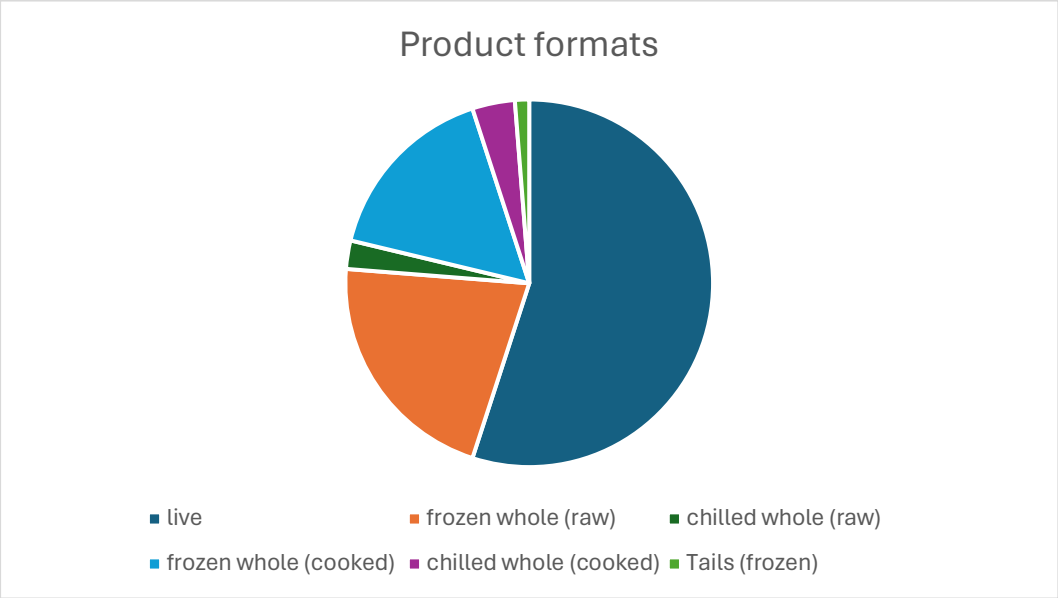
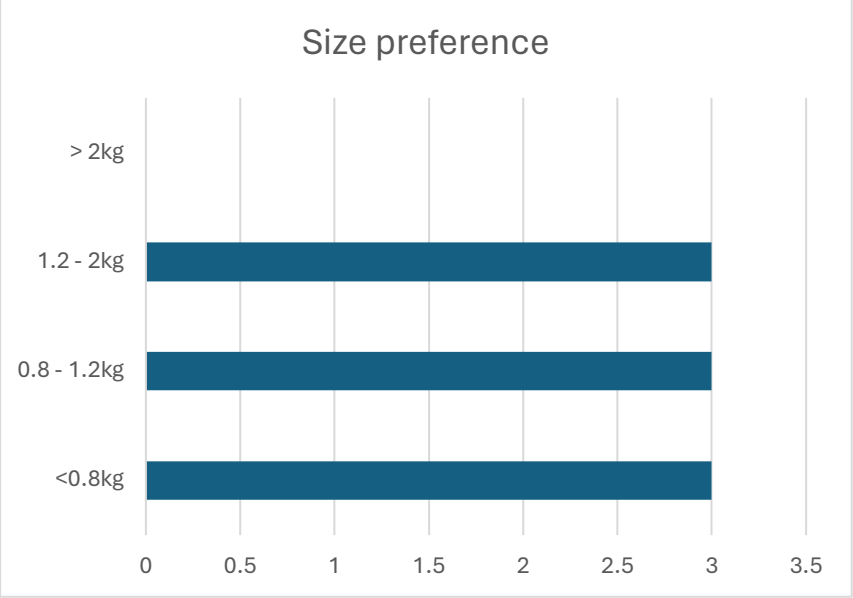


Figure 2: Size preferences (n=6)



Participants were asked to identify and rank the importance of factors influencing their decision to purchase and/or use rock lobster on their menus. Questions were asked unprompted (Fig 3) and prompted (Fig 4)

Figure 3: What factors influence the purchase of lobsters - unprompted (n=6)

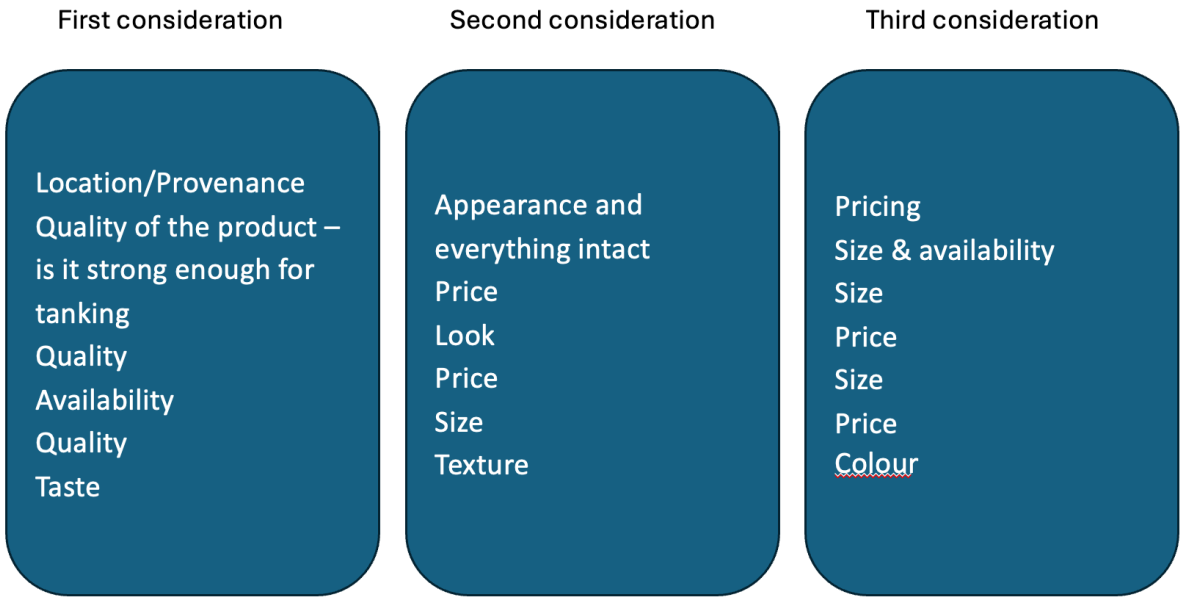
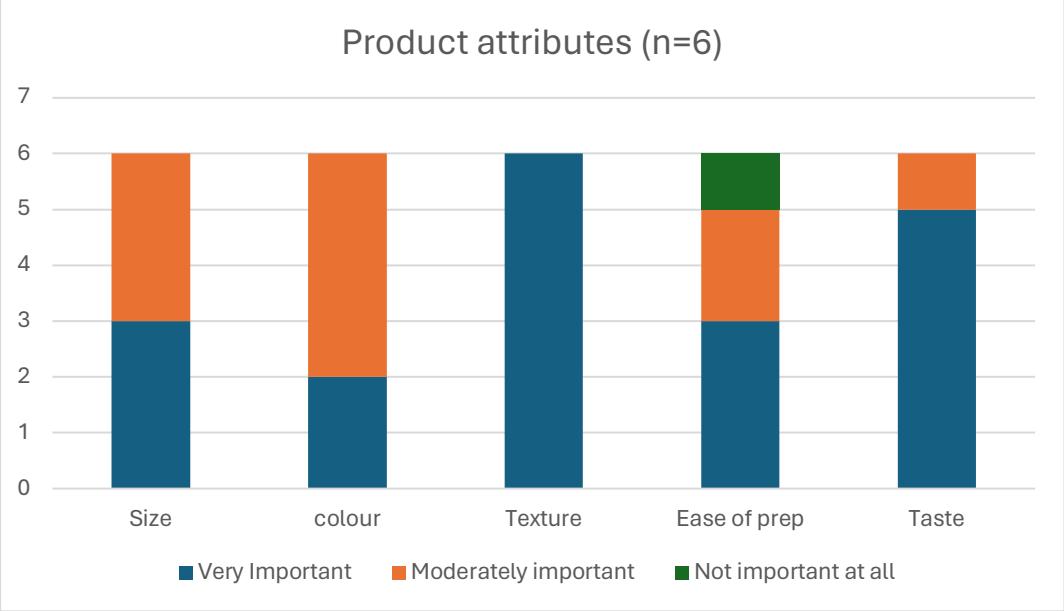


Figure 4: What factors influence purchase decisions - prompted

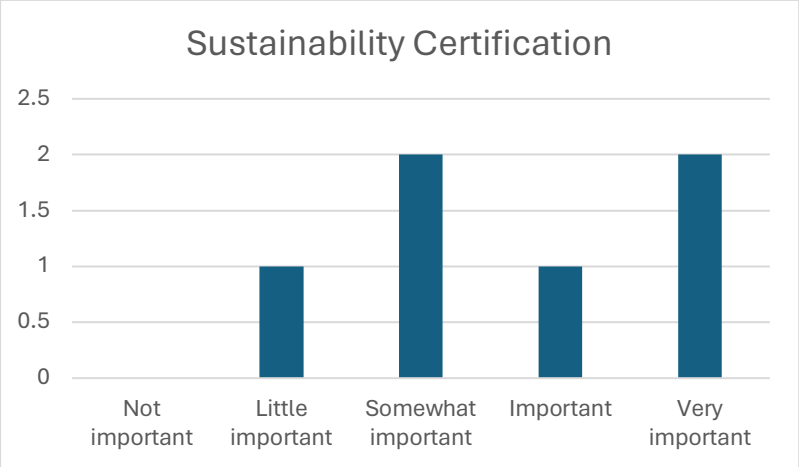


While quality was the most mentioned attribute in the unprompted question, this provides few clues as to what " quality " means. The prompted question found a unanimous vote for texture and taste as very important, or important with some divergence of opinions on the other factors. These results confirm the findings of other demand research undertaken in this project.

We were particularly interested in how important colour is, and in this case, it was considered important but not as highly rated as texture and taste. This is also somewhat consistent with previous research, and while diverse colours are seen as a distinguishing feature of tropical rock lobster, there doesn't appear to be a consistent view of what that means.

With the emergence of discussions about climate change impacts on and from food production and supply chain activities, we asked the participants about their views on the importance of sustainability certification (Fig 5). Again, quite consistent with previous research on this topic.

Figure 5: Importance of sustainability certifications (n=6)



Interview Findings

Part 2 of the survey (see Attachment 2) involved in-depth participant interviews. We were looking to understand :

- General impressions of the hatchery and grow-out processes and opinions on the words and language we should and shouldn't use when communicating about this.
- Whether there were any significant discernible differences between the wild and "closed lifecycle farmed product that we should be aware of and potentially address

Full details of the comments and suggestions are in Attachment 3 but are summarised here in Fig 6 and 7 below.

Fig 6: Initial impressions of farm tour participants (n=6)

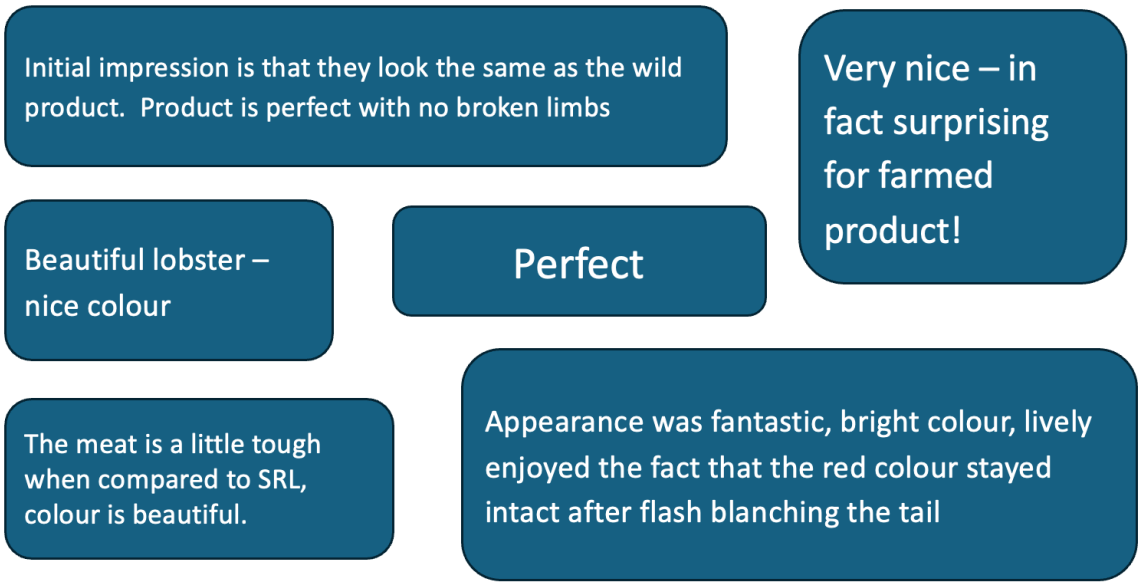


Fig 7: Taste and Texture comments from farm tour participants

The initial impressions of the process and the taste testing were highly positive for all cuisine styles tested. The consensus was that the product is ready for commercial trials in stores and restaurants once the supply stabilises. Care needs to be taken on how to describe the process as it was thought that the word “farmed” might have some negative connotations and devalue the product, which could be sold as a premium product.

The comment comparing tropical rock lobster taste and texture to Southern Rock Lobster was in relation to sashimi-style preparation. SRL is universally accepted as the most suitable for this sashimi style. Further testing this issue would require a larger sample and conducting a taste test directly comparing wild-caught Tropical Rock Lobster and Ornatas farmed products.

One feature that might also distinguish the Ornatas product from the wild-caught product is the length of the feelers. Again, this would need to be tested with a larger sample size, directly comparing Ornatas product with wild-caught product AND investigating whether the end user cares about this aspect.

Conclusion

The findings of this small focus group of experienced rock lobster users (chefs, retailers, wholesalers) demonstrate that the Ornatas product is very acceptable to the market. More work needs to be done to directly compare the product to the wild-caught product, using larger sample sizes and cuisine styles.

Recommendations and Next Steps

1. Undertake ongoing taste testing trials comparing against wild-caught products.
2. Work with supply chain partners to trial different market communications about the Ornatas production process, including how to describe it. Continue communicating with farm visit participants to gauge response as these messages and materials are developed.
3. Train Ornatas team members on harvesting and packing products for transport to retail and food service establishments. This could be done through the partnership with Torres Straits Seafood (based in Cairns) and focus initially on supplying produce very locally (Townsville and Cairns)

Attachments

Attachment 1: Farm Tour information

Attachment 2: Survey and IDI protocol

Attachment 3: IDI results

Welcome to the Ornatas secret....

FARM TOUR

5 - 6 March 2024
Ornatas Tropical Rock Lobster Facility
Toomulla Beach, Northern Queensland

ornatas

For more information visit: www.ghanemgroup.com.au

Jake Nicholson

Executive Chef, Ganhem Group

Jake Nicolson is the executive chef of the Ghanhem group. They have the following diverse venues

- Blackbird Bar and grill (steak and seafood) www.blackbirdbrisbane.com.au/
- Blackbird events (Riverview events/function)
- Donna Chang (modern Chinese with live tanks https://www.donnachang.com.au/
- Boom Boom room (Japanese) https://www.theboomboomroom.com.au/
- Iris rooftop (Spanish) https://irisrooftop.com.au/
- Bisou Bisou (French) https://bisou-bisou.com.au/
- Hotel X (hotel/ function)
- Modern Vietnamese opening in August

Jake has decades of experience, going from London to fine dining in Melbourne. He has now been in Brisbane with this group for 10 years this year. With the diversity of venues, he will be very up-front about quality, sizing, and pricing.
For more information:

For more information visit: www.the-ville.com.au

Nicholas Redsell

Executive Chef, The Ville

Nicholas Redsell is the executive chef from The Ville Townsville, https://www.the-ville.com.au/, which has several venues, including:

- Miss Song Modern Asian
- Quarterdeck – bar/bistro
- The Palm house – Tropical
- Splash Bar – Bar/ Bistro
- Spin Café – café
- Sports bar – bar
- Functions/ catering
- Opening Steak house, Japanese extensions TBC

Three chefs from The Ville will accompany Nick:

- Arie Prabowo
- Yukio Ozeki
- Indika Wijerantha

For more information visit: www.instagram.com/thefishgirl

Max & Francoise Pantachini

Preston Fresh

Max and Francoise Pantachini established retail and wholesale operations in Cairns in 1993. Preston Fresh is now the largest Seafood Retail Shop in Cairns. They bring a large component of their hospitality background into their work, having great respect for their produce and a passion for food creation.

The company started off as a two-person operation. Within five years, Max and Francoise had built up the company and employed over 45 staff and had five blue trucks supplying seafood to over 200 customers ranging from Cape York to Townsville as well as Interstate and International.

They also opened a retail shop opposite the James Cook University for 5 years. All of this took place whilst both their daughters where under the age of 5.

Throughout the years, Max and Francoise have built a reputable name for themselves in the seafood industry. In fact, they supplied seafood to her Majesty the Queen Elizabeth II on her visit to Cairns in 2002.

For more information visit: https://customseafood.com.au/

Damien Gan

Director, Custom Seafood Distributors

Damien Gan has supplied the hospitality and catering industry with some of Australia's finest seafood for the past 16 years. They wholesale live, fresh and frozen seafood.

Supplying Brisbane, Gold Coast, Sunshine Coast, Melbourne, Sydney, Canberra, Perth and South Australia with some of Australia's and the World's finest Sashimi, Fresh, Frozen and Live seafood. From state of the art purpose built 2000m2 Safe food accredited facility. Encompassing temperature controlled processing room, Live holding tanks, on-site freezer and cold rooms.

Custom Seafood has partnered with some of the best restaurants and chefs that Australia has to offer. Resident seafood experts, Damien and Grant have a range of knowledge and skill that is often sought-after to assist with planning menus and quantities to maintain client satisfaction, efficiency and profitability.

For more information visit: www.crcna.com.au

Sarah Doherty

Acting CEO, CRCNA

The Cooperative Research Centre for Northern Australia (CRCNA) Sarah Docherty is the acting CEO of the CRC for Northern Australia.

The CRCNA is investing $75m of Commonwealth funds over ten years to support industry-led research collaborations. As part of the Ornatas commercialisation process the CRCNA has invested in the partnership with Ornatas and the Fisheries Research and Development Corporation to trial grow-out techniques and understand market demands and drivers.

For more information visit: **www.ornatas.com.au**

Scott Parkinson

CEO, Ornatas

Scott Parkinson is the CEO of Ornatas.

He is passionate about science and the sea. He has worked in aquaculture for over 30 years across research and development, commercial operations and sales.

Scott is dedicated to a sustainable future for aquaculture, building the skills and expertise of the Ornatas team and engaging with communities to reach this goal.

Tony Barton

General Manager, Ornatas

Tony is the Ornatas General Manager. His role is to oversee core services that enable our workplace to function efficiently and out team to thrive.

He is also responsible for business administration and risk management. Tony has a collaborative approach, working with his team and across all levels of the business to build the best processes and ensure the right resourcing is in place to get everything done safely and effectively.

Jennifer Blair

Research & Development Manager, Ornatas

Jennifer Blair oversees the commercialisation of the lobster hatchery technology developed by UTAS at our Toomulla Beach site.

As our Hatchery and Research and Development Manager, Jen manages special projects.

She leads our strategic research and partnerships while also focusing on establishing Ornatas' grow-out operations, which has become a top priority for the company's future.

John Breen

Farm Manager, Ornatas

John Breen has over 18 years of farm management experience.

He is perfectly suited as our Farm Manager, having managed and guided the production planning and commercialisation of nursery and grow-out operations of the Tropical Rock Lobsters at our Toomulla Beach site.

Sandra Infante Villamil

Research & Project Manager

Sandra is our Project Manager for the CRCNA project.

As our Research Officer she participates in the scientific aspects of the project, including experimental design, data analysis and communication.

She also contributes to implementing our biosecurity and TRL health monitoring plans, which all underpin the provision of quality juveniles for raft grow-out research.

www.instagram.com/thefishgirl

Umar Nguyen

Director, The Fish Girl

Umar Nguyen known as "the Fish Girl," is a prominent figure on Instagram, but her impact goes far beyond social media. Her drive and enthusiasm and innovative marketing and engagement strategies have yielded impressive results for her clients and the seafood industry.

What sets Umar apart is her hands-on approach. She goes directly to the source, collaborating closely with producers, whether they are involved in wild-caught or farmed seafood. This on-ground experience allows her to gain invaluable insights into every facet of the seafood supply chain, from fishing and harvesting to processing, logistics, and distribution. Her previous work as a chef in some of the world's top seafood restaurants further enriches her understanding, uniquely qualifying her to design and execute sales and marketing campaigns that deliver tangible benefits to all involved.

Honey & Fox

www.honeyandfox.com.au

Jayne Gallagher

CEO & Co-Founder, Honey & Fox

CEO and co-founder of Honey & Fox, a specialist food marketing agency. Honey & Fox works with fishers, farmers, and specialty food manufacturers to create and grow premium brands in Australia and internationally.

Our tailored services include market research, strategy, creative and communications services.

Jayne has led the market research and development team currently focusing on the market demand and drivers.

Helen Johnston

Creative Director & Co-Founder, Honey & Fox

Helen leads the Honey and Fox branding, communications, and digital marketing team.

Helen's main focus is on elevating brands to premium, and creating products and platforms that are easy to use and result in customers feeling value from their interactions with a brand, product, or service.

Helen loves helping our clients connect with their markets by bringing their authentic provenance stories to life.

Honey & Fox

Ornatas Farm Visit & Product Tasting Feedback Form

March 2024

Thank you for agreeing to participate in this research. This interview is part of a project being conducted by Honey & Fox and Ornatas under a project funded by the CRC for Northern Australia.

This project aims to help Ornatas better understand market demands and drivers for tropical rock lobster to develop strategies that meet those needs.

Ethical considerations are important to us. This research is confidential; you and your company will not be identified in the research project. We would like to record a short interview with you to assist with the data analysis process. If you agree to this, you are welcome, at points during the recording, to ask us to cease recording at any time during the interview.

Can we record the interview?

If you have any questions or concerns about this research, please do not hesitate to contact us: team@honeyandfox.com.au or phone Jayne on +61 438336712

Business Name	
Business Type	
Business Location	
Name and Position	
Contact Details	

Part 1: General Questions about Lobster

Instructions: When responding to these questions, please refer to your experience with Lobster generally

1. **Do you currently work with Lobster, if so which species and where is it sourced from?**

2. **What is the most popular lobster product that you handle? Why do you think it is the most popular?**

3. **What are the three most important factors (with 1 being the most important) you consider when purchasing lobsters?**

1	
2	
3	

4. **What does quality mean to you in relation to Lobster?**

5. **How important is consistency in size, colour, and texture to you when purchasing lobster?**

Product attribute	Very important	Moderately important	Not important
Size			
Colour			
Texture			
East of preparation			
Taste			

6. **Can you describe your ideal Lobster?**

7. **In what form(s) do you normally purchase your Lobster (estimate is fine)?**

_____ % live
_____ % frozen whole raw
_____ % chilled whole raw
_____ % frozen whole cooked
_____ % chilled whole cooked
_____ % other, please specify

8. **How important is the provenance or origin of the Lobster to you?**

☐ Not at all important
☐ Little importance
☐ Somewhat important
☐ Important
☐ Very important

9. **What is your ideal size for a whole lobster?**

☐ <800g
☐ 800-1.2kg
☐ 1.2-2kg
☐ >2kg

10. **Is sustainable packaging important to you?**

☐ Not at all important
☐ Little importance
☐ Somewhat important
☐ Important
☐ Very important

11. **When deciding who/where to purchase from how important is sustainability certification to you?**

☐ Not at all important
☐ Little importance
☐ Somewhat important
☐ Important
☐ Very important

Honey & Fox

Ornatas Farm Tour and Taste Testing In-depth Interview Results

March 2024

Participants

Business Name	Platinum Providores (The fish Girl)
Business Type	Sales & Marketing (domestic)
Business Location	Brisbane
Name and Position	Umar Nguyen
Contact Details	0401 966588

Business Name	Custom Seafood Distributors
Business Type	Seafood Wholesale
Business Location	Brisbane
Name and Position	Damien Gan
Contact Details	0413673888

Business Name	Preston Fresh Seafood
Business Type	Wholesale/Retail
Business Location	Smithfield Cairns
Name and Position	Max and Francoise (owners

Contact Details	0458037230

Business Name	Ghanem Group
Business Type	Hospitality Group Restaurants
Business Location	Brisbane and Melbourne
Name and Position	Jake Nicolson Executive Chef
Contact Details	0413823093 jake@ghanemgroup.com.au

Business Name	Terasu Japanese Restaurant - ARDO
Business Type	Restaurant
Business Location	Townsville
Name and Position	Yukio Ozeki
Contact Details	0492824021

1. **Initial Impressions: What are your first impressions of the Ornatas Rock Lobster in terms of appearance and quality compared to wild-caught lobster?**

Perfect
Initial impressions is that they look the same as the farmed product. Product is perfect with no broken limbs
Beautiful lobster – nice colour
Very nice – in fact surprising for farmed product
Appearance was fantastic, bright colour, lively enjoyed the fact that the red colour stayed intact after flash blanching the tail
First time to see such a facility – it looks great – the challenge is that the meat is a little tough when compared to SRL, colour is beautiful.

2. **Sensory Attributes: How would you describe the texture, flavour, and aroma of the Ornatas Rock Lobster**

Sashimi: Clean although might prefer to be done in thinner slices
Boil – firm, salty flavour can come through
Grilled – a little tough/firm
Salt & Pepper – deep fried, firm, flaky

Texture is firm without being too firm. Taste profile is clean with a slight clean after taste. Amazed at the sweet flavour that boiling the product brought out.

Texture is amazing, flavour and aroma are very good

In fact pleasantly surprised, love it, great texture, unusual for a farmed product to be so close to wild

For sashimi – would need more texture without being tough

3. **How versatile is Ornatas Rock Lobster to cook with? How does it compare to other lobsters you have cooked with?**

Very versatile

Extremely versatile – with the mild flavour it will soak up anything you put with it

The same as other lobsters

Very easily used in variety of cooking methods, easy to remove meat from the tail, shell not too firm, doesn't over cook too easily

4. **Culinary Potential: What types of dishes or culinary applications do you believe would best showcase Ornatas Rock Lobster at its best?**

Sashimi and boiled (based on today's tasting)

Sashimi, lobster roll topped with caviar

Same as other lobsters

Halved and grilled, definitely Asian flavours and cooking techniques, wok stirfried

5. **Market Needs Adaptation: Given the ability to potentially modify size, colour, and texture, what specific product characteristics do you believe would make the Ornatas lobster appealing to your customers?**

Consistency and set food cost – for planning menus

The story

I felt it hit the mark for everything

Bright colour, shell intact and unbroken, locally produed, good meat to shell ration

6. **What factors (eg taste, sustainability, novelty) do you think would drive interest in Ornatas Rock Lobster and why?**

Circular economy
Location – locally produced

Solar panels on the carpark roof
Consistency in taste from controlled feed

The story of how it came to be, then the sustainability and then the taste

Would be good if they can sell a lobster plate size

Sustainability, novelty, regularity of supply, versatility in size – can grow to order

A catchy name or unique story "the world's greatest painter"

Consider a cooking competition where contestants have to produce a best dish to drive interest and enthusiasm

7. **Is there anything in the Ornatas farming process that raises any concerns or issues that we may need to address when we are marketing and promoting Ornatas Rock Lobster to the market?**

When talking with retail customers – not using the word "farmed"
I think people will question the sustainability of the feed and probably waste product management.

8. **Do you have any other comments, thoughts or suggestions to help us market and promote Ornatas Rock Lobster?**

No – thank you for the tour. It was really helpful to see
Please contact me when you are ready to sell
The factory was amazing, well-organised, clean and very efficient. Thank you for having us (maybe do a plate-size lobster?)
Lots of thoughts were shared on the day, so I feel that we have achieved a lot – thank you very much for sharing.
I think understanding why it's a superior product is important
Look towards food festivals such as Taste Port Douglas or Noosa Food and Wine